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Table of Content

Table of Content

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African Journal of Agricultural Research

Opinion

Launching a Startup Business in the Agribusiness Market: The Process of becoming a Successful Entrepreneur

Conclusions from the CEIBS (China Europe International Business School) program at Harvard for Business Women in the Agribusiness Market

Maria Luisa del Pozo Lite

Innovation needs entrepreneurship and entrepreneurship needs innovation

Last December 2017, Prof. Marisa del Pozo Lite (Complutense University of Madrid) was invited to give a session as member of the International Advisory Board of CEIBS-Africa to a group of 40 African Business Women in the Agribusiness Market – as part of the Alumni Reunion Agenda of CEIBS-Africa Program held at Harvard University in 2017.

Getting a venture underway in the agribusiness market is often easier than keeping it going and growing. At each major stage from start-up to sustainable success, entrepreneurs face tough questions about shifting gears, making major changes, and letting go of people, partners, and products. For new businesses, inability or unwillingness to change can land them in the statistics about high failure rates at the five-year mark. For non-profits, clinging to the past can lead to marginality and stagnation. To keep an enterprise on track in the agribusiness market while facing the often-pleasant challenge of growth requires making sometimes-painful adjustments in these five areas:

THE PEOPLE

One of the hardest questions is when to change the people: not just individually, but the whole mix. Founders often start with friends and true believers who work hard because of zeal for the cause or hope for future returns. They occupy multiple overlapping roles. But do the people with single-digit badge numbers or members of the founding generation have the skills the organization needs as it creates routines and requires depth in every specialty? Who can make the cut? A winery I knew from its beginning kept the original group longer than the business could afford, and loyalty got in the way of bringing in experienced people "above" the people who felt they were founders and thus privileged to call the shots. Raise a glass to courageous leaders willing to tell people they must either grow or go.

Finances

Whether the original source of funds is venture capital or venture philanthropy, an investor base or a donor base, each growth phase challenges organizations to shift assumptions and thus change practices. Perhaps investors expect customers to take over as funders of growth by paying more (or paying at all); a challenge that agribusiness companies face now. Non-profits also outgrow friends-and-family angels or local sources and must find sustainable revenue and capital sources. How do you move from being discretionary nice-to-have in a portfolio to essential-to-fund? Where are the new sources appropriate to a new, larger size? A multi-site non-profit went from local businesses close to the founding city to national funders in government and foundations to a revenue model replicable in every site through ongoing school budgets on a fee-for-service basis.

Partners and allies

The best organizations are attuned to the need for key external relationships that provide resources and support. At the same time, entrepreneurs do not want to be captive to the needs and desires of their first distribution partners, component suppliers, source of talent, or marketing allies. It is tricky to know how to nurture and draw benefits from key partners without being subsumed by them or subject to damage if they stumble and, at the same time, add to a partner set without creating conflicts. Which partners should be downplayed or replaced as the organization grows? How can key relationships be managed to lessen dependence while seeking new, more relevant, allies? And with growth comes the need for entirely new types of relationships

Organizational culture

Are you making explicit what the organization stands for in tangible ways that can be transmitted and endure? Are you on guard against drifting away from the culture? Numerous studies, including my own, show that an emphasis on organizational culture is associated with continuing excellence. Values, stories, artifacts, and rituals provide a source of identity that makes the organization feel the same, in pursuit of the same mission even while everything else changes. Culture provides internal glue. As an organization grows, what was once informal must be documented, codified, memorialized, and passed on to new people. Savvy entrepreneurs ensure that their organizations are built to last by stressing culture. At every stage, they invest in preserving fundamental values and principles while adding new iconic stories that reflect them.

Outcomes and impact

What results are being produced, for whom, and are these sufficient? In the beginning it is enough to show that it can be done at all, to address a good cause or to prove that something works in a handful of markets. In the next phase, you might look at growth indicators; we did more this year than last year: Are you making a difference that makes the venture more essential?

Ventures that go from proof of concept to "permanent" player have become icons, household names, or must-have players because they can show differentiated user, recipient, or national benefits: that they have impact not just on their immediate customers but on the entire industry. We all know that success provokes imitation. As the organization grows, its distinctiveness gets harder to maintain but often, many in and around the organization come to believe that existence is a sufficient sign of importance; a trap particularly for non-profits. Asking the "so what if we were not here?" question about making a difference can provoke soul-searching and strategy change.

The bottom line, in addition to the challenges of innovation to ensure new offerings and new capabilities, is that the entrepreneurs and organization founders in the agribusiness marketplace must also be alert to the ways that the organization itself changes as a result of growth. It is important to anticipate those developments and ask the five big questions at every stage in order to get ahead of change and master it.

HOW DO WE UNDERSTAND THE PROCESS OF ENTREPRENEURSHIP?

The pursuit of opportunity without regard to the resources is currently controlled by:

Entrepreneurial management

1) Strategic orientation
2) Commitment to opportunity
3) Commitment of resources
4) Control of resources
5) Management structure
6) Compensation schemes

Processes of entrepreneurship

1) Pursuit of opportunity
2) Rapid commitment and change
3) Multistage decision making
4) Using other people's resources
5) Managing through networks and relationships
6) Sharing the value created

Skills and traits of successful entrepreneurs

1) Ability to create the illusion of stability
2) Organize great teams amidst chaos
3) Risk management
4) Tolerance for ambiguity
5) Attention to detail
6) Long time perspective
7) Endurance

Risks to successful organizations

1) Inflexibility from successful past practice
2) Bureaucratic processes
3) Isolation
a) From new technology
b) From customers
4) Hubris generation over commitment
5) Short term thinking and greed

Requirements to be a leader

1) Deep industry knowledge
2) Patience
3) Willing to make hard choices
4) Dry powder
5) A little luck

Why management must change

This is shown in the table below:

Old world	New world
Hard to go wrong	Hard to go right
Rules clear	Rules unclear
Local competition	Global competition
Level financing field	Widely varying financing
Customers with personal standards ties and commitment	Customers with NO ties and commitment
Imperfect information	Almost instant data

Entrepreneurial process

1) See patterns as they form
2) Prepare a plan
3) Take action
4) Revise
5) Retrench
6) Recommit

Requirements for an entrepreneurial culture

1) Perceiving opportunity at all levels
2) Wanting to pursue opportunity at all levels
3) Helping people to believe that they can succeed

Increasing perception of opportunity

1) Jobs with real time market connections
2) Responsibility for broadly defined objectives
3) Respect for many talents
4) Valuing change

Making people want to pursue opportunity

1) Pursuit must be in the individual's self-interest
2) Making plan cannot be the goal
3) Changing plans cannot be the same as failure
4) Risk of failure for the individual must be shared with the organization and the culture

Making people believe that they can succeed

1) Short term slack
2) One "no" cannot be fatal
3) Multi-staged resource allocation
4) Sharing of resources

Keys for management in the age of uncertainty

1) Building solid teamwork out of strong individuals
2) Reward functional excellence
3) Emphasis on responsibility not authority
4) Accepting the right kinds of failure
5) Insuring continuous adaptive change
6) No surprises control

What can the boss really do?

1) Select, motivate and reward the right people
2) Ensure resources are available
3) Facilitate coordination
4) Rationalize decision premises

Remove the impediments to performance!

Issues critical to all innovative entrepreneurial cultures

1) Resource mobility
2) Reinvestment in community
3) Joy in the success of others
4) Valuing change

Take home points

1) Entrepreneurship is a process not a person!
2) Pursuing opportunity beyond the resources currently controlled is key
3) Pursuing opportunity is different from managing resources.
4) Culture, systems and management behavior can make or break the entrepreneurial mold

Factors and cultural differences in entrepreneurship

1) Why innovation and entrepreneurship now?
2) Defining entrepreneurship
3) Entrepreneurs and managers
4) Entrepreneurial cultures

Why are innovation and entrepreneurship needed now?

1) Technological change
2) Economic change
3) Political change
4) Social change
5) Psychological change

Traditional definitions of entrepreneurship

1) Risk taker
2) Founder
3) Innovator
4) Capitalist
5) Scoundrel

Innovation and entrepreneurship

1) Not just start-ups
2) Not just something that has never been seen or done before
3) Not just small companies
4) Not just technology based ventures
5) Not the high flying fast living unscrupulous promoters

What is entrepreneurship?

Entrepreneurship is an approach to management that starts with opportunity

What is an opportunity?

1) A desired future state that is different from the present
2) A belief that achievement of that state is possible

Opportunity

1) Depends on the environment
2) Depends on access to required resources
3) Depends on the person
4) Depends on timing

OPPORTUNITY NEVER STANDS STILL

The climate for innovation

1) Higher costs
2) More demand for value
3) Consumers feeling pinched
4) Intense competition
5) Fewer but bigger players in most domains
6) Rapidly evolving regulation

"Build a better mousetrap and the world will beat a path to your door" Ralph Waldo Emerson

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Full Length Research Paper

Effect of soil compaction on aerial and root growth of *Erythrina velutina* Willd.

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Compaction can be a triggering factor for a process of soil degradation and formation of degraded areas. Understanding plant development in soils compacted is of fundamental importance, since they allow to identify species able to resist the limiting condition of the compaction, being able to be indicated to recover degraded areas that have compacted soils as limiting factor. The objective of this study was to evaluate the initial aerial and root growth of *Erythrina velutina* in soils subjected to different levels of compaction. The experiment was conducted in a greenhouse located at UECIA/UFRN. A Yellow Latosol of Frankish-sandy texture, from an area of the Jundiaí Agricultural School, was used in pots formed by three overlapping polyvinyl chloride (PVC) rings, 10 cm in diameter and 25 cm in height, with the central ring being compacted. The experimental design was a randomized block design, with six replications, and four levels of soil compaction (1.35, 1.45, 1.60 and 1.80 kg dm^{-3}) were tested, and the following variables: diameter, height, number of leaves, dry mass of shoot and root system in each layer of the columns. The physical impediment in subsurface altered the aerial growth of the seedlings of *E. velutina* being this reduction more expressive for the dry mass variable of the aerial part. In relation to the root system, *E. velutina* showed to be a susceptible species to the effects of soil compaction and morphological changes were observed in the roots in soils with a density greater than or equal to 1.45 kg dm^{-3}.

Key words: Root system, soil density, soil management, recovery of degraded areas.

INTRODUCTION

There are currently alot of study soil compaction by effecting limiting root growth of plants. Plants are the source of life in the living world. They perform many ecological functions in their environment, and they shape the life of living things in the environment where they live. The life of living things in the world is directly or indirectly dependent on plants (Cetin, 2013, 2016; Sevik and Cetin, 2015; Guney et al., 2016; Yigit et al., 2016a, 2016b). The

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ability of plants to fulfil their functions primarily depends on the availability of appropriate climatic and edaphic conditions (Cetin and Sevik, 2016; Guney et al., 2016, 2017; Cetin et al., 2017a). Therefore, soil is one of the absolutely necessary conditions for plant existence, which is essential for the life of living things.

The soil is defined as “the part of the solid earth that has been altered by the loosening of the earth, humus formation and chemical decomposition, by the transport of humidification and chemical decomposition products” (Cetin and Sevik, 2016; Sevik et al., 2016, 2017a, 2017b; Cetin, 2017; Cetin et al., 2017b; Turkyilmaz et al., 2017; Kuscu et al., 2018). However, when it is examined in detail, the soil is a very complex structure and the biological and biochemical process in the soil is the basis of the terrestrial ecosystem (Cetin and Sevik, 2016; Sevik et al., 2016, 2017a, 2017b; Cetin, 2017; Cetin et al., 2017b; Turkyilmaz et al., 2017; Kuscu et al., 2018). In this respect, it is very important to examine the structural change of the soil and to determine its relation with the plant.

Some studies shows that it examined the change of the soil structure in the forests according to the tree species. An attempt to determine some soil characteristics based on tree species and depth of soil was made within the scope of the study. Soil is important for forest and landscape. Enzymes in the soil structure ensure that they are alive in forest areas (Sevik and Cetin, 2015; Cetin, 2013, 2016; Cetin and Sevik, 2016).

Intensive farming, inadequate management and removal of vegetation such as excessive exploitation of timber resources and the uncontrolled use of fire, have generated several physical problems attributed to the soil world wide (Bargali et al., 1992,1993a; Joshi et al., 1997), which combined with the low capacity for regeneration of Caatinga soils, can lead to increased susceptibility environment and desertification and formation of degraded areas (Melo et al., 2008; Sousa et al., 2012). According to Azevêdo and Azevêdo (2012), the recovery of degraded areas has become a constant concern of researchers in the Northeast region of Brazil.

In the Caatinga, the environmental degradation originating from agricultural activity has been increasing. Potentiated by the edaphoclimatic characteristics of the environment, its irrational use has contributed to the biome degradation (Campos et al., 2016).

According to Tavares et al. (2008), soil compaction can be a triggering factor for degradation and degradation of degraded areas, by reducing water infiltration rate, limiting root growth of plants, lower availability of nutrients, and reduce the avalable pore space in the soil resulted in poor soil microbial population which adversally affct the the litter decomposition (Bargali, 1996; Bargali et al., 1993b, 2015).

An area can be considered degraded, when in the event of a strong impact, loses its capacity of resilience, necessitating mitigating measures to revert to the adverse condition (Martins, 2013). Therefore, solutions should be adopted based on the diagnosis of the degraded area and from the type of degradation. In the case of compaction, in addition to commonly used silvicultural practices, one of the alternatives may be the use of plants that have the aggressive root system (Tavares et al., 2008).

The mulungu (*Erythrina velutina* Willd.), is a native species characteristic of the Caatinga Biome. A pioneer in successional stages, it presents great ecological importance in the colonization of secondary areas, being very used in the recovery of degraded areas (Lorenzi, 2002; Matos and Queiroz, 2009). However, there are no studies that address its development in compacted soils.

It is possible that certain species, due to their specificities can even in compacted soils, establishing, improving the physico-chemical properties of the soil and retrieving the quality of same (Reinert et al., 2008).

These specificities help in the selection of species capable of growing under adverse conditions, and may be indicated to recover degraded areas that have compacted soils as a limiting factor. According to Reichert et al. (2003), soil densities critical to plant development on frankish-sandy soils vary from 1.6 to 1.8 kg dm^{-3}. In no-tillage, a system often used to recover degraded areas, a compaction and an evident problem, always being observed to a greater or lesser degree of intensity (Cardoso and Coutinho, 2013).

With these facts, the overall objective of the study was to evaluate the effect of soil compaction on the initial aerial above-ground and root growth of *E. velutina* in a Yellow Latosol of frankish-sandy texture, submitted to different levels of compaction.

MATERIALS AND METHODS

To conduct this experiment, Yellow Latosol of frankish-sandy texture, from the forest experimental area of the Agricultural School of Jundiaí of the municipality of Macaíba-RN was used.

To obtain a higher soil homogeneity, soil portions of the subsurface layer (B horizon) were collected at a depth between 20.0 and 40.0 cm. Afterwards, the soil was dewormed, air-dried and sieved in 2.0 mm mesh, homogenized and sub-samples were analysed for chemical and physical properties (Table 1).

Based on the chemical analysis, soil correction was not performed due to the high value of base saturation and the absence of aluminium, and only basic fertilization was performed for the installation of the urea, simple superphosphate and potassium chloride in the amounts of 150, 300 and 100 mg dm^{-3}, respectively.

The experiment was conducted in a greenhouse located at the Academic Unit Specialized in Agricultural Sciences (UECIA), Federal University of Rio Grande do Norte, Macaíba-RN. The greenhouse is lined with 1.0 mm mesh nylon mesh and transparent glass fiberglass (minimum temperature of 24°C and maximum of 38°C).

The experimental design was a randomized block with six replicates, containing five seeds per experimental unit, and the effect of soil compaction on the initial growth of *E. velutina* at the densities of 1.35 (no compacted layer), 1.45, 1.60 and 1.80 kg dm^{-3}.

The experimental unit was represented by a polyvinyl chloride

Table 1. Chemical and physical characterization of the soil used in the experiment.

Chemistry	Value
pH in water (1 : 2,5)	5.78
P ($mg.dm^{-3}$)[(1)]	2.00
K^+ ($mg.dm^{-3}$)[(1)]	268.00
Na^+ ($mg.dm^{-3}$)	132.00
Ca^{2+} ($cmol_c.dm^{-3}$)[(2)]	1.18
Mg^{2+} ($cmol_c.dm^{-3}$)[(2)]	0.40
Al^{3+} ($cmol_c.dm^{-3}$)[(2)]	0.00
H+ Al ($cmol_c.dm^{-3}$)[(3)]	0.75
SB ($cmol_c.dm^{-3}$)	2.83
CEC (T) ($cmol_c.dm^{-3}$)	3.58
V (%)	79.05
Physical	
Sand ($g.kg^{-1}$)	688
Clay ($g.kg^{-1}$)	180
Silte ($g.kg^{-1}$)	132
F.C (%)	9.04
P.W.P (%)	7.03

[(1)]Mehlich-1extractor; [(2)]KCl1mol.L^{-1}extractor; [(3)]Calcium acetate 0.5 mol L^{-1}extractor at pH 7.0. SB: Sum of bases; CEC: cation exchange capacity at pH 7.0; V: base saturation; F.C: field capacity; P.W.P: permanent wilting point.

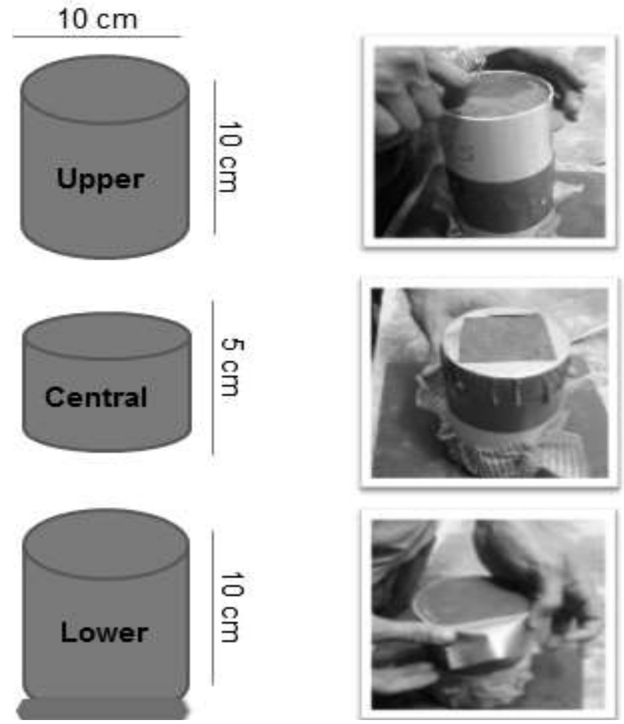

Figure 1. Schematic illustration of the experimental unit used in the experiment.

(PVC) column 10 cm in diameter and 25 cm in height. It was composed of three layers (upper, central and lower), the height of the upper and lower layers being 10 cm; while the central layer was 5 cm, joined by adhesive tape. To close the base of the bottom layer of the column, a multipurpose cloth was used, affixed with rubber alloys (Figure 1).

The upper and lower layers of the PVC column were composed of non-compacted soil; while the central one under soil subjected to four different soil densities. This compaction was done in 2.5 and 2.5 cm layers of soil by blows with a metal plunger, the soil being pressed in the PVC column until the volume corresponding to the desired density inside the central layer of the column.

To avoid root development by the compacted PVC-soil interface (points of least resistance to penetration), the methodology described by Müller et al. (2001), where adhesive tape was placed about 2.0 cm wide, folded from the periphery to the center of the upper surface of the central ring, avoiding the development of the roots contiguous to the wall (Figure 1).

Fifteen days after emergence of the seedlings, thinning was done, leaving only one plant per experimental unit until the end of the data collection, performed 60 days after emergence of the seedlings. Irrigation was performed daily with a graduated cylinder, using the volume of water corresponding to the soil field capacity.

After 60 days of seedling emergence, the height, the collecting diameter at the level of the soil, number of leaves and dry mass of the area and roots at each layer of the experimental unit were recorded. The layers of each column were separated with the help of a stylus, in the three corresponding parts. Then, the soil roots were seperated for each layer and washed out under running water using 1.0 mm sieves to avoid root loss. The shoots and roots were placed in a oven at 65°C for 72 h to determine the dry mass, using an analytical balance.

The data were compared by means of analysis of variance at the 5% probability level and the regression study, using the equation that best fit the untransformed data, using statistical program Assistat 7.7.

RESULTS AND DISCUSSION

The analysis of variance showed that, with the exception of the variable collar diameter (CD) that is not tuned to any regression model, all other variables analyzed were adjusted only to the linear regression model, indicating that the compaction attributed to the subsurface layer of soil interfere significantly in the initial growth *E. velutina* (Table 2).

In relation to the variables height, number of leaves and shoot dry mass of the aerial part, it was observed from the analysis of the regression that, the treatment composed by uncompacted soil showed the best means, having the increase of the density of the soil to the maximum level of compaction, resulting in a reduction of 13.86, 19.72 and 37.85% in the growth of the aforementioned variables, respectively, when compared with the treatment which composed of uncompacted soil, evidencing that the presence of compacted layer in subsurface promotes detriment of the variables that would continue to respond negatively to the compaction effects under larger soil densities (Figure 2).

The results found for the species *E. velutina* corroborate those found by Ribeiro et al. (2010), where the increase

Table 2. Variance analysis and mean values of collar diameter (CD), number of leaves (NL), height (H), shoot dry mass (SDM), and root dry mass in the upper (RDM.U), central (RDM.C) and lower (RDM.L) column of *Eritryna velutina* undergoing different levels of compaction.

SV	DF	Average squares						
		CD	NL	H	SDM	RDM.U	RDM.C	RDM.L
Treatments	3	3.118	8.041	34.621	8.948	0.304	0.047	0.117
Linear	1	6.745^{ns}	21.675*	84.168*	21.819*	0.632*	0.141**	0.348**
Quadratic	1	0.158^{ns}	1.041^{ns}	15.843^{ns}	0.996^{ns}	0.250^{ns}	0.00002^{ns}	0.004^{ns}
Cubic	1	2.451^{ns}	1.408^{ns}	3.852^{ns}	4.029^{ns}	0.310^{ns}	0.001^{ns}	0.00007^{ns}
Blocks	5	1.828^{ns}	1.441^{ns}	10.110^{ns}	2.507^{ns}	0.304^{ns}	0.006^{ns}	0.013^{ns}
Residual error	15	3.322	3.508	18.354	2.783	0.338	0.003	0.004
CV (%)	-	14.83	17.91	14.04	27.52	25.95	26.18	27.67

SV: Source of variation; GL: degrees of freedom; CV: coefficient of variation (%). ns: not significant, **significant at the level of 1% and *significant at the 5% probability level by the F test.

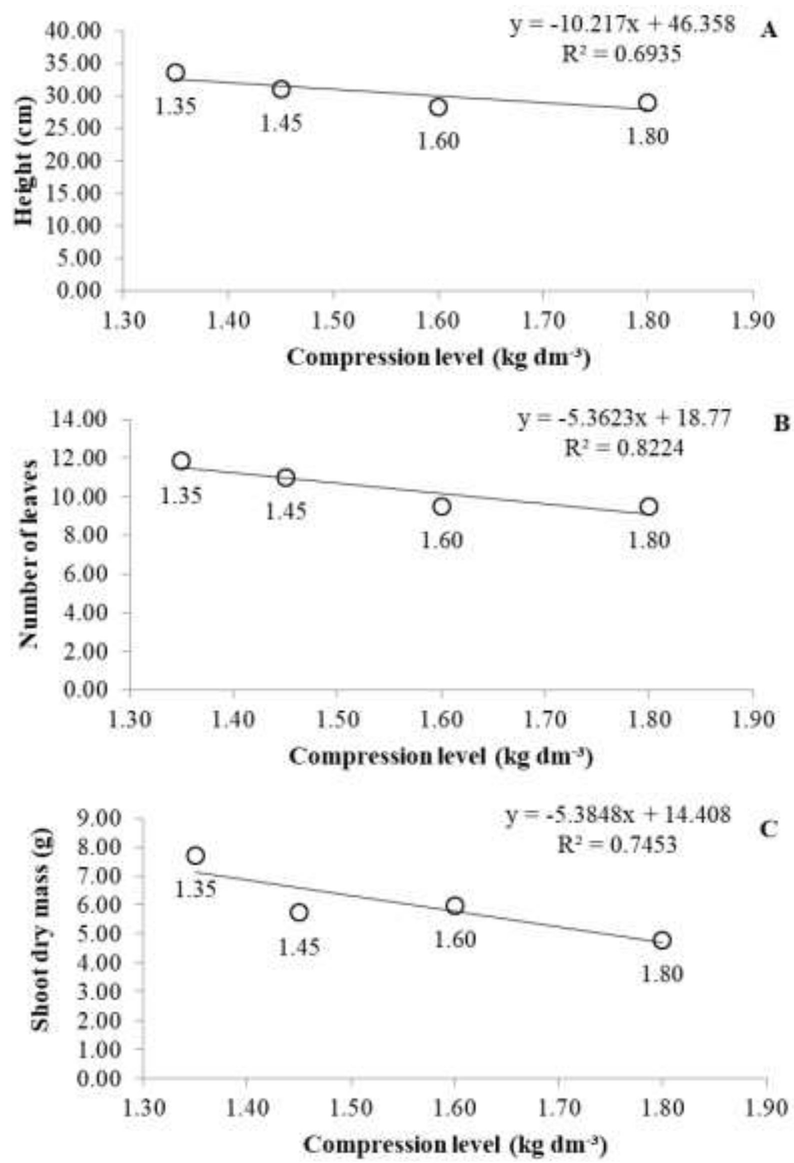

Figure 2. Height (A), number of leaves (B) and shoot dry mass (C) of *Erythrina velutina* plants due to different levels of compaction.

of soil density promoted restrictive effect on growth *Eucalyptus grandis* for the variables height and shoot dry mass, regardless of the type of soil evaluated.

Borges et al. (1986), evaluating the effect of soil compaction in the initial growth of three species of eucalyptus, observed that for the species tested, the negative regression coefficient showed that even small, there was a decrease in the shoot dry mass of the seedlings.

Ohland et al. (2014), also report a linear decreasing trend of the variable height for the species *Jatropha curcas* L., being observed a reduction in plant height of approximately 25% to the highest level of compression tested (1.64 kg dm^{-3}), when compared with lower density (1.08 kg dm^{-3}).

Santos et al. (2012), evaluating the development of *J. curcas* L, depending on the soil density, observed the effect of regression analysis, a linear decrease for all variables analyzed, except for the number of leaves that have increased in function of compaction. Already Pereira Junior et al. (2012), evaluating the growth of roots and shoots of *Moringa oleífera* under conditions of compacted soil, report no significant difference among treatments for the variables diameter, height, shoot and root dry mass, being considered a species relatively resistant to compaction, for not presenting significant effects both on initial growth airspace, as well as in the roots.

In relation to the development of the root system, it was observed that the physical constraint imposed by the compacted layer significantly influenced the root production in all the columns of the vessels and the data was adjusted to the linear regression model (Figure 3).

In the upper column, a linear increase of the root dry mass variable was observed in the aforementioned layer, where the soil density increased at the highest compaction level (T4 - 1.80 kg dm^{-3}) promoted an accumulation of 18.83% of the variable, when compared with the control treatment, composed of uncompacted soil.

Through visual observation, it was verified that the linear increase of roots in the upper column of the experimental unit partially restricted the root system expansion in depth, thus promoting the accumulation of roots in the upper column and the folding of roots (Figure 4).

Reinert et al. (2008), evaluating the physical quality of a Dystrophic Red Latosol after the cultivation of cover plants, reported a normal growth of roots until the limit of density of 1.75 Mg m^{-3}. In the range between 1.75 and 1.85 Mg m^{-3}, restrictions were suggesed on growth, combined with the deformations in the morphology of the roots in medium grade, while above this density, the deformations became more significant, with extensive thickening of the roots, deviations in vertical growth and concentration on the most superficial layer.

The results differ from those observed by Silva et al. (2012), who valuated the effect of compression on the species of *J. curcas* and *Crambe abyssinica*. According to the authors, the physical impediment caused in the subsurface layer in a Red Latosol of medium texture, was not enough to stop the root growth vertically.

Other visual aspect observed was the considerable reduction of the macroporosity of the soil in relation to the increase of the soil compaction, hindering the growth of thick roots and creating a physical layer of resistance to root penetration, thus restricting the vertical expansion of the pivot root along the ground from the density of 1.60 kg dm^{-3}. Moreover, after the processing of the roots, it was possible to observe that the presence of a compacted layer soil densities equal to or greater than 1.60 kg dm^{-3}, caused morphological changes in the growth of the pivot root (Figure 5).

The macroporosity is an important characteristic in soil aeration and drainage of water, as well as the main route to the root growth of plants. When poorly structured or deformed, or can inhibit the development of plants (Camargo and Alleoni, 1997). According to Neves et al. (2005), it is necessary to adequate number of channels that are large enough to allow the roots may penetrate vertically in the ground and develop.

Ohland et al. (2014) evaluated the influence of soil density in the initial development of *J. curcas* L. and reported that densities between 1.50 and 1.64 kg dm^{-3} were considered critical for the development of the species, being noticed, also restricting the expansion of the turnable root in deeper layers, making the most superficial root system in relation to the increase of soil density. The inhibition of root pivoting growth was also observed by Reinert et al. (2008) soil densities greater than 1.85 Mg m^{-3}.

Limitation of pivotal root growth and surface root development in the soil may be a relevant problem for tree species such as *E. velutina*, where inhibition of the root system may reduce the stability of the trees, causing them to be tilted under field conditions.

In central and lower columns of the experimental units, the effect observed was the detriment of the variables, where a large decrease was found in the production of roots of *E. velutina* in the function of increased soil compaction. The highest level of compression tested observed drastic reductions of 64.00 and 75.58% in the production of roots dry mass in the columns above, respectively, being that higher densities to continue promoting the detriment of variables. This restriction has been criticized even in smaller densities of soil as the treatments T2 (1.45 kg dm^{-3}) where a reduction in the production of roots dry mass was 25.5% at the core layer and 31.78% in the layer below the compressed and T3 (1.60 kg dm^{-3}), where the reduction was 39.5 and 55.81%, respectively.

Borges et al. (1986), analyzing the responses of eucalyptus seedlings to soil compaction, observed that the growth of roots of *Eucalyptus camaldolese, Eucalyptus tereticornis* and *Eucalyptus grandis* was nil in

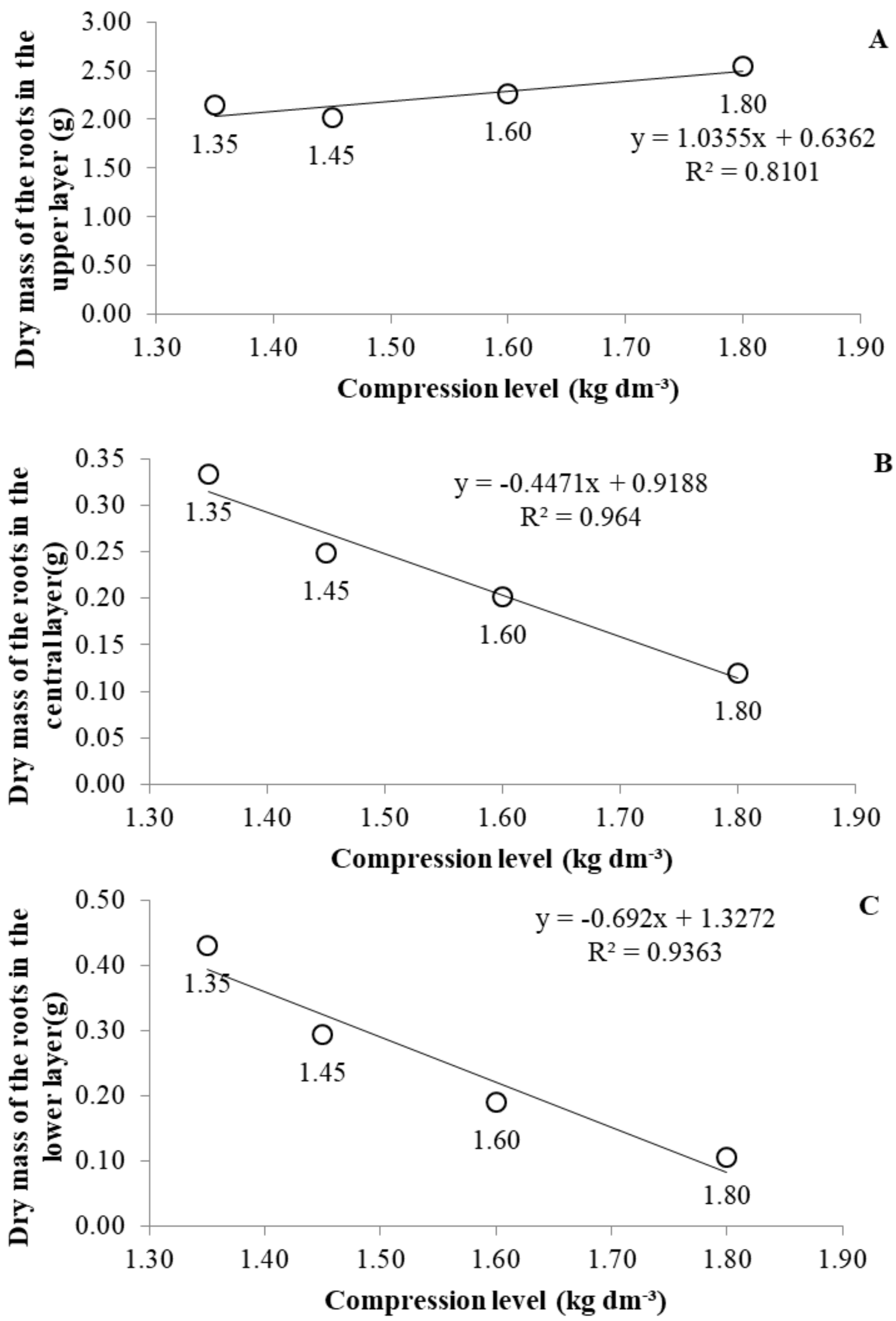

Figure 3. Root dry mass of *Erythrina velutina* in the upper (A), central (B) and lower (C) layers as a function of different levels of soil compaction.

a Dark Red Latosol with 72% of clay soil for densities higher than 1.15 g cm^{-3}, characterizing the species as sensitive to soil compaction.

Differently, from what has been reported in the present study, Pereira Junior et al. (2012) report inexpressive effects of the soil compaction on root growth of *M. oleífera*.

In fact, the pattern of growth of *E. velutina* was not maintained when the species was submitted to the effect of compaction. Such a pattern of growth can be a reflection of morphological changes caused in the root system under conditions of compression, which when associated to lower soil volume exploited, promoted a possible poor nutrition, reflecting in their productivity.

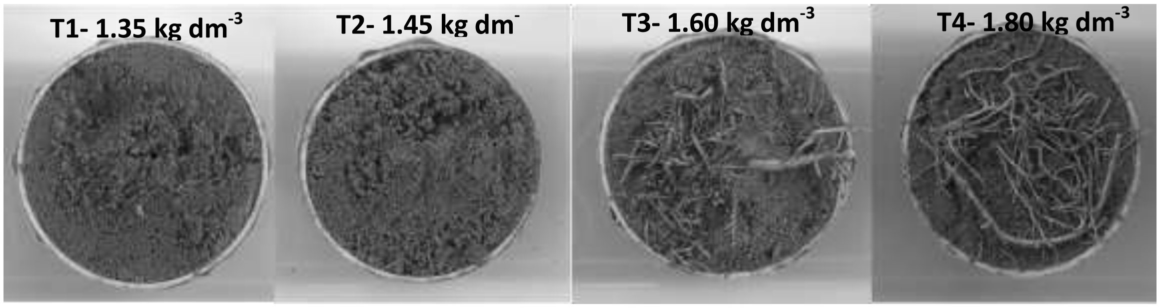

Figure 4. Internal view of the upper layer of the PVC column after sectioning of the experimental units by using a stiletto. Distribution of the apparently unchanged root system in soil without any compacted layer (A), with a compacted layer at a density of 1.45 kg dm^{-3} (B) and 1.60 kg dm^{-3} (C), and apparent accumulation of roots in the surface layer due to subsurface soil compaction at a density of 1.8 kg.dm^{-3} (D).

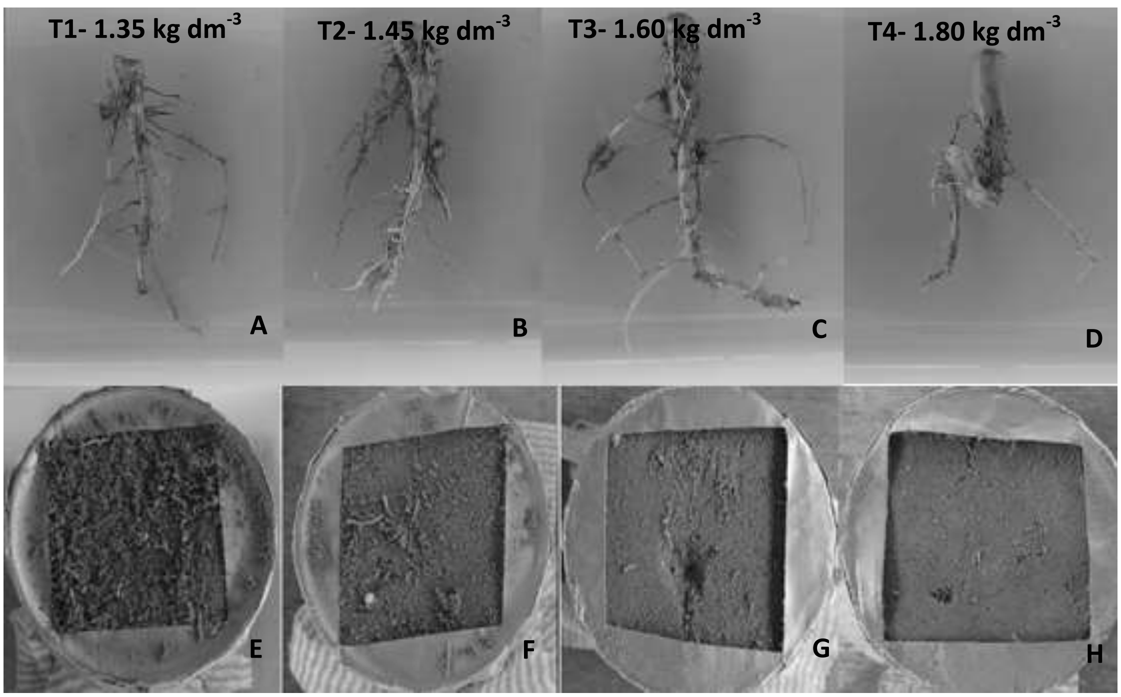

Figure 5. Pivotal root located in the upper layer of the experimental unit after root rooting, apparently without any morphological changes (A and B) and negative effect on the pivoting root as a function of the compacted layer, morphologically altering its structure (C and D). The presence of pivoting root in the central layer under uncompacted soils (E) or when compacted the density of 1.45 kg dm^{-3} (F) and negative effect of the compaction under the pivoting root limiting its growth in the central layer the density 1.60 kg dm^{-3} (G) and 1.80 kg dm^{-3} (H), respectively.

For Alvarenga et al. (1997), physiological and morphological changes of roots make them less efficient in the absorption of nutrients, in this way, the extent of absorption of nutrient is achieved more slowly, consequently, the plants grow less, due to the smaller quantities of absorbed nutrients. According to the same author, the amount of nutrients such as phosphorus, calcium, magnesium, and nitrogen in leguminous, was reduced due to increases in the level of soil compaction.

The root system may be the first component affected by compression, because the development of fine roots, where there is a higher rate of absorption of nutrients is impaired (Reichert et al., 2007). This pattern was applied for the present study, having in view that the effects of the soil compaction were much more expressive for the variables related to the root growth than for the variables related to the growth of the plant.

It is believed that in a major period of evaluation, the results would be more expressive, that is, the plant will suffer more accentuated effects of compaction. In this way, further studies were suggested with the species under field conditions, where the evaluation period may be greater.

Finally, an important consideration to be made is that *E. velutina* proved to be a susceptible species to soil compaction effects. Although, considering a pioneer species and recommended in the recomposition of secondary areas, decreasing linear reductions was observed for the variables of the aerial part of the seedlings and a severe impact on the vertical growth of the root system, noticed from equal or higher soil density to 1.45 kg dm^{-3}, indicating that the species should not be recommended for recovery of degraded areas with compacted soil as a limiting factor, unless mechanical practices such as scarification can be made to soften the physical constraint imposed by compacted soils.

Conclusion

The increase in soil density of 1.35 to 1.80 kg dm^{-3} in subsurface layer affects the aerial and root growth of *E. velutina* in the initial phase of plant development, which may be observed in the reeled pivoting root from soil density equal to or greater than 1.60 kg dm^{-3}, not being a species recommended for recovery of degraded areas that have compacted soil as a limiting factor.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Application of cow manure and inorganic fertilizer in one season and carryover of effects in sesame on tropical ferruginous soils

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The aim of this research was to find whether *Sesamum radiatum* growth requires cow manure and nitrogen-phosphorus-potassium (NPK) fertilizers. We examined the survey vegetable over two growing seasons on ferruginous soil: from June to December in 2009 (Season 1) and 2010 (Season 2). We used cow manure (0, 20, 30 and 40 t˙ha^{-1}) and NPK 10:10:20 (0, 50, 100 and 150 kg˙ha^{-1}) during season 1 and no amendments during season 2 to determine if application of amendments would carry through to a second season as partially described by effects on soil and yield. A randomized complete block designed through three replications is performed. Parameters such as pH, Organic carbon, Nitrogen, phosphorus, exchangeable bases (Ca^{2+}, K^+, Mg^{2+}), cation exchange capacity, base saturation and yields were identified. Cow manure significantly affected height, leaves yields while NPK had a lowly significant effect on total yield, stems yield and base saturation and highly significant effect on leaves yields, sum of bases and cation exchange capacity. Both cow manure and NPK fertilizers significantly affected magnesium and organic carbon. 20 t·ha^{-1} of cow manure and 100 kg˙ha^{-1} of NPK produced the best yield in the second season and our results opened up encouraging perspectives in using magnesium, organic or inorganic fertilizers for sesame growth. The outcomes provided important prerequisite for effective cultivation of the survey leafy vegetable and it is expected to be applied in research for cultivars suitable to modern planting systems and food purposes.

Key words: Amended soil, unamended soil, yield, growing seasons, leafy vegetable.

INTRODUCTION

Soil fertility issues were serious concerns in West Africa. Local farmers amended their soils with plant and green manure. These organic resources could not improve soil fertility as they were decomposed due to high temperature, torrential rainfall or high solar radiation and were insufficient in quantity to meet the requirements of

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crops on most farmlands. In tropical ferruginous soils, some incomplete weathering of primary minerals occurred alongside the predominance of kaolinite minerals. Three types of ferruginous soils are encountered in Benin: depleted tropical ferruginous soils, slightly leached tropical ferruginous soils and leached tropical ferruginous. These soils account for 65% of the total area of the country, have a low water holding capacities and various levels of nitrogen, potassium and phosphorus (Padonou et al., 2015). A low level of organic matter is usually found in ferruginous tropical soils (Cissé et al., 2016). Factors limiting agricultural use of sandy soil include nutrient deficiencies, acidity, low water storage or poor physical attribute (Boutchich et al., 2018). Farmers used to grow annual crops on this soil, but few leafy vegetables. Sesame species were adapted to tropical agro-ecological conditions, easy to cultivate and were not very demanding in terms of inputs (Watson and Eyzaguire, 2002).

The leafy vegetable sesame used in this report, *Sesamum radiatum* Schum. and Thonn, was a traditional leafy vegetable consumed by households in Benin. It has antimicrobial, anticataract and antioxidant activities (Seukep et al., 2013; Dzoyem et al., 2014); it is found in various habitats and is not an endangered species (Bedigian, 2004). The leaves and oil of this vegetable are consumed as staples food (Konan et al., 2013). The leaves contain a high level of proteins, iron, magnesium, low levels of crude fat and fiber, and moderate levels of anti-nutrients oxalate, phytic acids and tannins (Nanloh et al., 2015). This species of sesame is a neglected and underused leafy vegetable revealed in Benin by an ethno-botanical survey (Agbanpké et al., 2014, Dansi et al., 2012). Deficiencies in micronutrients such as iron iodine, zinc and vitamin A are well known to affect children and childbearing women (Bain et al., 2013). Joy et al. (2014) estimated that micronutrients deficiency risks in Africa were highest for calcium, zinc, selenium, and iodine, while the risks for copper and magnesium deficiencies were low. These deficiencies could be eliminated by changing diets to include a greater diversity of nutrient-rich foods and useful leafy vegetable. Besides, leafy vegetables are known to contribute to a healthy diet (Baldermann et al., 2016). In Sub-Saharan Africa countries, there are a wide variety of plant species that can be consumed as leafy vegetables (Lagnika et al., 2016), but half of these countries do not meet the vegetables intake of at least 400 g person^{-1} day^{-1} as advocated by the World Health Organisation (Tata-Ngome et al., 2017). Many traditional leafy vegetables were simply being lost leaving behind just a fraction of the most popular varieties of vegetables that were less nutritious and more dependent on pesticides and fertilizers (Dansi et al., 2008). These trends can have immediate consequences on the nutritional status and food security of the populations. Therefore, for their potential to be exploited to advantage there was a need to understand their growth requirement. Pour et al. (2013) reported that the growth, yield, and quality of leafy vegetable were affected significantly by organic and inorganic nutrients applied. *S. radiatum* was an important source of iron and copper (Agbanpké et al., 2015). This vegetable had a short growth cycle and produces high yields per season and was drought-tolerant adaptable to many soil types and intolerant to wet conditions (Lim, 2014). Most of the relevant studies on yield measurement were limited only to *Sesamum indicum*, one of the oldest oilseed crops cultivated worldwide (Amoo et al., 2017) and originated from Africa (Ram et al., 1990). This crop is in the same genus, *Sesamum*, as the survey leafy vegetable. Reports in literature, addressed the traditional cultural practices (Dansi et al., 2012) and genetic characterization with markers (Adéoti et al., 2011) associated with *S. radiatum* cultivation. On the other hand, a polysaccharide extracted from *S. radiatum* leaves was analysed for its binding properties in tablet formulation (Allagh et al., 2005) or as matrix formers for sustained release tablets (Nep et al., 2016). However, no information about a fertilization requirements of this sesame species is available.Whether fertilization was relevant to scale-up its production on ferruginous soils remained to be established. The proper method for optimal exploitation of our sesame species potential has to be identified.

The objectives of this study were: (1) evaluate physicochemical properties of a tropical ferruginous soil in absence of fertilization, (2) identify organic and inorganic fertilizer treatments that benefit leafy vegetable yields, and (3) assess effects of unamended soil on leafy vegetable growth.

MATERIALS AND METHODS

Survey area and soil sampling analysis

The ferruginous sandy, slightly acidic, a soil of the survey site (Table 1) subjected to a rainy season extended from April to October and a dry season from November to March was analyzed over a 2-year experimental period. The geographical location is 8°1'59" North, and 2°28'59"East. A field trial was conducted between July and December 2009 (Season 1) and 2010 (Season 2). Soil samples were collected from 20 cm depth on June (before experiment), and December (after harvesting) for both seasons and placed in a sterile plastic bag, labeled and transported to the laboratory for determination of their chemical properties. Soil particle size analysis was performed following ISO international standard of soil particle-size analysis by destroying the soil organic matter with hydrogen peroxide using the dispersing sodium agent hexametaphosphate. After drying and weighing, a physicochemical analysis was performed on air-dried soil samples. Three samplings were made before field trial and means were computed (Table 2). Analyses were based on 1:2.5 ratios for pH-water. Other measured variables were: OC identified with Walkley-Black dichromate method adapted by ANNE (AFNOR X31-109) and based on the reduction of $K_2Cr_2O_7$ and subsequent determination of the unreduced dichromate by an oxidation-reduction titration with ammonium sulfate (Landon et al., 1991). Ca^{2+}, Mg^{2+} and K^+ were

Table 1. Texture and physical properties of the tested soil.

Soil property	Values
Texture	Sandy
Sand content (≥0.02 mm)	89.75%
Silt content (0.002 mm-0.02 mm)	2.10%
Clay content (≤0.002 mm)	8.15%

Table 2. pH, nutrient content and some physical characteristics of the survey ferruginous soil before field trial of Season 1 in 2009.

pH-water	pH-KCl	Total N (%)	OC (%)	OC:N	Avail P (mg/kg)	Total P (mg/kg)	Ca^{2+} (meq/100 g)	Mg^{2+} (meq/100 g)	K^{+} (meq/100 g)	S (meq/100 g)	CEC[a] (meq/100 g)	V (%)
6.74±0.37	6.15±0.08	0.07±0.02	1.02±0.06	16.07±1.39	17.3±0.53	47.78±0.78	1.28±0.39	1.12±0.09	0.39±0.00	2.79±0.01	10.33±1.09	27±0.24

[a]CEC : Cation exchange capacity.

determined by the Atomic Absorption Spectrophotometer. Total nitrogen was identified by Kjeldahl digestion (Jackson, 1958); exchangeable bases was extracted with ammonium acetate solution at pH 7 and determined by atomic-absorption spectrometric as described by Metson (1974), available phosphorus extracted with Bray I method (Bray et al., 1945), and total phosphorus as determined by Duval (1963). The granulometric analysis was determined by the method of Robinson's pipette (1922).

Yields and growth parameters assessment

Seeds supplied locally and by the "6AVG" project during 2009 were sown directly into 7 m^2 (1 × 7 m) seedbeds with 3 seeds per hole. Seedbeds, settled on ferruginous soil were covered with *Panicum maximum* grass, which after removal, was replaced with a 15 cm shade made of *Eleais guineensis* leaves. No amendments or phytosanitary treatments were applied. Seedlings were watered daily with 22 L of water. Soils of the plots were dug-up, mixed, turned, harrowed and leveled before receiving seedlings. To identify effects of amendment application time on sesame growth, NPK 10:10:20 fertilizer at 0, 50, 100 and 150 NPK $kg \cdot ha^{-1}$ via a double ring method and cow manure at 0, 20, 30 and 40 $t \cdot ha^{-1}$ rates were applied before plant establishment in season 1. For season 2, no amendments were used. Twenty-one-day-old seedlings were transferred to 80 equal-sized plots every 2 m^2, with a row to row spacing and plant to plant spacing of 1 m. After 5 days, plants were thinned to 1 seedling per hole. Each plot received 32 seedlings distributed into 4 lines, with 160,000 $plants \cdot ha^{-1}$. Seedlings that did not straighten 48 h later were replaced. All plants were watered daily with 22 L of water until they were firmly established in the soil. Plots were hand weeded and hoed once weekly. For yield estimation, plants were harvested from 2 middle rows. Three harvestings were realized on 0.48 m^2 plots. The first harvesting occurred 6 weeks after transplanting and 2 others occurred at 9 WAT and 12 WAT; yields were summed for each season. Fresh leaves separated from fresh stems were weighed immediately after cutting to determine total biomass. The dry weight of leaves and stems were performed by placing samples in a forced air oven at 80°C for 72 h. Numbers of fully expanded leaves, plant height from the soil surface to the apical bud, and a number of vegetative branches containing at least 4 fully expanded leaves were collected at 2WAT and 4WAT during Season 1 and 2 to determine short-term effects of fertilization on growth. The experimental design was a randomized complete block containing 16 treatments: 0 CM (Cow manure) and 0 NPK or control, 0 CM and 50 NPK, 0 cm and 100 NPK, 0 CM and 150 NPK, 20 cm and 0 NPK, 20 CM and 100 NPK, 20 CM and 50 NPK, 20 CM and 100 NPK, 20 CM and 150 NPK, 30 CM and 0 NPK, 30 CM and 50 NPK, 30 CM and 100 NPK, 30 CM and 150 NPK, 40 CM and 0 NPK, 40 CM and 50 NPK, 40 CM and 100 NPK or 40 CM and 150 NPK.

Data were subjected to the analysis of variance of the ANOVA procedure in SAS (ver. 9.4, SAS Inc., Cary, NC. USA). For differences amongst treatments, results of statistical tests were considered statistically significant when the P value of the ANOVA procedure was less than or equal to 0.05. Interactions involving NPK and cow manure significant with the ANOVA procedure were further analyzed via 2-way analysis using least squares means and means were separated with the Least Significant Differences of Tukey's. Average was calculated for a parameter with non-significant interrelationships.

RESULTS

Description of the rainfall regime and temperature variations

The rainfall of the survey area was 1164.4 mm. Three peaks were observed: 1 peak in March (94.5 mm) and May (122.9 mm) during the long rainy season and 1 peak in August (273 mm)

Table 3. Means values of rainfall and temperatures on the experimentation site from 2009 to 2010.

Parameter	Month											
	January	February	March	April	May	June	July	August	September	October	November	December
Year 2009												
Rainfall (mm/day)	67.50	19.40	64.20	114.60	76.90	148.20	216.10	97.10	82.00	48.90	27.20	0
Temperature (°C)												
Maximum	35.50	37.20	37.30	34.10	33.70	32.20	30.70	29.10	30.50	32.30	34.60	37.20
Minimal	22.0	24.20	24.50	23.30	23.60	23.00	22.40	22.30	22.20	22.70	22.10	23.20
Year 2010												
Rainfall (mm/day)	0	0.90	94.50	69.70	122.90	80.20	99.40	273.00	227.70	152.80	43.30	0
Temperature (°C)												
Maximum	37.50	39.10	37.80	36.30	33.60	32.90	30.60	30.20	31.00	32.60	34.40	36.00
Minimal	23.50	24.60	25.40	24.90	24.10	23.80	22.70	22.40	22.50	22.70	23.00	22.00

during the small rainy season (Table 3). The lowest average rainfall was 43.3 mm·day^{-1} in November. The trials were subjected to a range of rainfall from 0 to 148.20 mm·day^{-1} during season 1 and 80.20 to 227 mm·day^{-1} during season 2. The temperature peaked on July 2009 and dropped in August, and rose again until December for season 2 (2010). Maximum monthly average values from June to December were between 29.10 and 37.20°C for Season 1 and between 30.60 and 36°C during season 2. Minimum monthly average values from June to December were between 23.00 and 23.20°C for Season 1 and between 22 and 22.70°C during Season 2. Therefore the leafy-vegetable was more watering during season 2 than in Season 1.

Effect of mineral and organic amendments on sesame yields

The interrelationships among NPK fertilizers, cow manure, and nutrient variables are assessed in Table 4a and their mean square or degrees of freedom are presented in Table 4b. In these tables, NPK fertilizers affected the total yield, leaves yield and stems yield while cow manure affected only leaves yield. The interaction between the growing seasons and CM affected the number of branches and the height of four weeks after transplanting. Interestingly, the number of branches of 4WAT was also affected by NPK fertilizers. The growing seasons affected the total yield, total leaves yield, total stem yield, two weeks and four weeks after transplanting yield, respectively. For non-significant interrelationships among growing season, cow manure and NPK fertilizers, we performed average. Therefore, for season 1, the overall averages leave values of 2WAT and 4WAT were respectively 13.31±2.11 and 33.69±4.03. Considering 4WAT, height average is 14.99±1.38 cm and the average of branches is 11.35±2.25. Conversely, in Season 2, our analysis showed that overall averages leave values of 2WAT and 4WAT were 18.49±3.27 leaves and 101.72±10.07 leaves, respectively, while the height of 4WAT was 29.29±4.30 cm. The average number of branches was 6.52±1.06.

In Table 5, of all treatments investigated, only 0 CM and 150 NPK, and 40 CM and 150 NPK had a total yield value below that of the control (0 CM and 0 NPK) at the end of Season 1. The highest total yield (23.41 t·ha^{-1}) or stems yield (8.78 t·ha^{-1}) was produced by 0 CM and 100 NPK treatment but the highest leaves yield came from 40 CM and 50 NPK (18.53 t·ha^{-1}). Relative to the control, the later treatment leaves yield increased by 53.14%. Conversely, the lowest total yield came from 40 CM and 150 NPK treatment. Here, the values reported were 17.72, 9.61 and 5.11 t·ha^{-1} for total yield, leaves yield and stem yield respectively. Interestingly, 20, 30 and 40 CM produced a close total yield rate during Season 1 that is higher than that of 150 NPK treatments but was lower than the total yield of 50 NPK or 100 NPK treatment. These results suggested a positive contribution of cow manure to sesame yield and the negative effect of NPK fertilizers at a rate of 150 kg·ha^{-1} on the total yields. During Season 2, the treatment 20 CM and 100 NPK ensured a higher total yield (21.65 t·ha^{-1}), total leaf yield (12.75 t·ha^{-1}) and

Table 4a. Analysis of variance table for cow manure and/or NPK fertilizer and growing season's effects on height, yield of sesame and number of leaves reported on ferruginous soil.

Parameter	Yield (t·ha^{-1})			Growth parameters			
	Total	Leaves	Stems	NL-2-WAT[a]	NL-4-WAT	NBra-4-WAT	Height-4-WAT
Manure (M)	0.0790	<0.0060	0.2582	0.5542	0.7994	<0.0001	0.0159
NPK (F)	0.0005	<0.0001	0.0010	0.1804	0.9637	0.0008	0.3987
Season (S)	<0.0001	<0.0001	0.0004	<0.0001	<0.0001	<0.0001	<0.0001
Interactions							
M×F	0.0028	<0.0001	0.0082	0.5813	0.1261	0.7535	0.2616
S×M	0.1811	0.6820	0.2927	0.0754	0.2032	0.0209	0.0038
S×F	0.0997	0.0060	0.1406	0.1303	0.4804	0.0589	0.6108
S×M×F	0.2090	0.0981	0.7114	0.4227	0.0919	0.3350	0.3476

ns, *, **, *** not significant or significant at P<0.05, P<0.01, P<0.001, least squares means analysis.
NPK = NPK fertilizers.
[a]NL-2-WAT and NL-4-WAT = number of leaves 2 and 4 weeks after transplanting in Season 1 and 2.
NBra-4-WAT = number of branches 4 weeks after transplanting in Season 1 and 2.
Height-4-WAT = Plant height 4 weeks after transplanting in Season 1 and 2.

Table 4b. Mean square and degrees of freedom table for cow manure and/or NPK fertilizer and growing season's effects on height, yield of sesame and number of leaves reported on ferruginous soil.

Parameter[a]		Yield (t·ha^{-1})			Growth parameter			
		Total	Leaves	Stems	NL-2-WAT[a]	NL-4-WAT	NBra-4-WAT	Height-4-WAT
Manure (M)	MS	20.743	12.536	2.703	17.677	171.051	47.095	118.606
	Df	3.000	3.000	3.000	3.000	3.000	3.000	3.000
NPK (F)	MS	59.611	43.837	12.030	42.323	47.297	25.036	31.833
	Df	3.000	3.000	3.000	3.000	3.000	3.000	3.000
Season (S)	MS	317.321	663.835	27.414	1073.814	185113.032	934.847	8222.929
	Df	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Interactions								
M×F	MS	35.310	13.822	8.369	29.162	1029.851	4.367	30.952
	Df	9.000	9.000	9.000	9.000	9.000	9.000	9.000
S×M	MS	14.668	1.378	7.839	74.802	725.820	31.754	149.928
	Df	3.000	3.000	3.000	3.000	3.000	3.000	3.000
S×F	MS	38.794	34.622	3.710	60.615	382.816	23.827	18.107
	Df	3.000	3.000	3.000	3.000	3.000	3.000	3.000
S×M×F	MS	24.878	13.257	4.291	32.111	814.702	4.714	33.905
	Df	9.000	9.000	9.000	9.000	9.000	9.000	9.000

[a]MS: Mean Square, Df: degrees of freedom of Table 4 data.

Table 5. Effects of cow manure and NPK fertilizers on yields parameters during the two growing seasons.

Cow manure	0				20				30				40			
× NPK	**0**	**50**	**100**	**150**	**0**	**50**	**100**	**150**	**0**	**50**	**100**	**150**	**0**	**50**	**100**	**150**
Total Yield1	17.40^{ce}	21.80^{ac}	23.40^{a}	16.60^{de}	19.20^{acd}	21.00^{ac}	22.10^{ab}	19.70^{acd}	19.10^{ace}	19.30^{acd}	19.20^{acd}	22.70^{a}	18.30^{bce}	22.90^{a}	22.30^{ab}	14.70^{e}
Yield LF1	12.10^{ce}	15.40^{ac}	15.60^{ac}	11.00^{de}	13.30^{ce}	14.80^{acd}	17.90^{ab}	13.70^{bce}	13.20^{ce}	14.20^{acd}	15.30^{acd}	14.70^{acd}	13.80^{bce}	18.50^{a}	15.30^{acd}	9.61^{e}
Yield ST1	4.94	5.46	8.78	5.50	5.87	6.22	5.64	6.01	6.37	6.35	5.48	7.99	5.12	6.17	6.98	5.11
Total Yield2	17.70	14.60	17.90	12.5	20.40	16.30	21.6	15.00	14.8	15.80	16.1	19.3	16.60	15.90	19.8	20.1
Yield LF2	11.00	9.07	10.40	7.73	11.9	9.48	12.8	8.95	8.93	9.76	9.34	11.20	9.68	9.91	11.30	11.9
Yield ST2	6.70	5.56	7.51	4.79	8.45	6.82	8.90	6.00	5.90	6.02	6.80	8.09	6.88	6.04	8.52	8.25

$^{a-e}$Means in a row without a common superscript letter differ ($P < 0.05$) as analysed by two-way ANOVA and the TUKEY test.
Total Yield1 = Total yield of Season 1, Yield LF1 = Yield leaves of season 1, Yield ST1 = Yield Stems of Season 1,
Total Yield2 = Total yield of Season 2, Yield LF2 = Yield leaves of season 2, Yield ST2 = Yield Stems of Season 2,
Total Yield = Yield leaves + Yield Stems. Yield is estimated in tons per ha (t·ha^{-1}).

Table 6. Effects of cow manure and NPK fertilizers on growth parameters during the two growing seasons.

Cow manure	0				20				30				40			
× NPK	**0**	**50**	**100**	**150**	**0**	**50**	**100**	**150**	**0**	**50**	**100**	**150**	**0**	**50**	**100**	**150**
NLF-2-WAT1	10.30	13.60	11.30	11.80	14.50	13.10	13	17.90	12.10	14	13.50	15.60	9.80	12.90	16.30	13.20
NLF-2-WAT2	22.10	23.50	16.70	14.60	17.30	20.30	17.60	13.20	12.40	17.80	17.70	20	20.60	23.5	18.60	19.90
NLF-4-WAT1	28.10	32.10	35.20	31.10	31.60	37.80	36.70	33.40	30	36.50	42	38.60	28.60	31	36.50	29.80
NLF-4-WAT2	134	111	69.40	104	94.50	102	90.80	95.20	77.80	96.20	109	107	115	105	124	91.6
Nbra-4-WAT1	6.80	9.00	10.40	9.40	8.60	12	13.60	12.60	11.60	13.60	14.80	13.80	10.40	12.60	12.80	9.60
Nbra-4-WAT2	7.30	7.15	5.50	5.25	6.25	5.75	5.75	5.00	5.45	6.90	8.70	6.75	8.05	6.85	7.45	6.15
Height1	12.10^{d}	14.40^{bd}	15.80^{abc}	13.10^{cd}	14.10^{bd}	15.30^{abc}	16.50^{ab}	15.60^{abc}	14.10^{bd}	15.80^{abc}	17.60^{a}	16.30^{ab}	13.30^{cd}	15.40^{abc}	15.80^{abc}	14.10^{bd}
Height2	31.10	29.40	24.30	28.00	26.80	27.70	26.30	22.80	24.30	29.20	30.90	30.60	36.40	30.90	39.40	30.40

Values are means, n = 5 per treatment group.
$^{a-e}$Means in a row without a common superscript letter differ ($P < 0.05$) as analysed by two-way ANOVA and the TUKEY test
NL-2-WAT1 = number of leaves 2 weeks after transplanting in Season 1,
NL-4-WAT1 = number of leaves 4 weeks after transplanting in Season 1
NL-2-WAT2 = number of leaves 2 weeks after transplanting in Season 2,
NL-4-WAT2 = number of leaves 4 weeks after transplanting in Season 2
Height1 = Plant height 4 weeks after transplanting in season 1, Height2 = Plant height 4 weeks after transplanting in Season 2.

stems yield (8.90 t·ha^{-1}) of all tested treatments of our survey. Conversely, the lowest total yield (12.52 t·ha^{-1}), total leaf yield (7.73 t·ha^{-1}) and total stem yield (4.79 t·ha^{-1}) were reported for 0 CM and 150 NPK treatments. These findings confirm the negative effect NPK fertilizers at a rate of 150 kg·ha^{-1} reported previously. In addition, only the yield of 30 or 40 cm was lower than that of 100 NPK treatments. 20 CM had a total yield higher than that of 100 NPK treatments. It appears therefore that cow manure had a sustainable positive effect only at a rate of 20 t·ha^{-1}. When this

Table 7a. Analysis of variance table for cow manure and/or NPK fertilizer and growing seasons effects on physicochemical parameters measured in 0-20cm depth on ferruginous soil.

Parameter [a]	pH water	pH KCl [a]	OC(%)	N(%)	OC:N	Avail. P	Total P	Ca^{2+}	Mg^{2+}	K^{+}	S	CEC	V
Manure (M)	<0.0001	0.0155	<0.0001	<0.0001	0.0097	0.0018	<0.0001	<0.0001	<0.0001	0.0034	<0.0001	<0.0001	0.1087
NPK (F)	0.6153	0.1466	0.1535	0.0302	0.0184	0.9722	0.0031	0.5127	0.0004	0.1613	<0.0001	<0.0001	0.0021
Season (S)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0227	0.0024	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Interactions													
M×F	0.4369	0.3051	0.0124	<0.0001	0.0254	0.1086	0.0328	0.9968	<0.0002	0.8059	0.0009	0.1579	0.2591
M×S	0.0010	0.8378	<0.0001	<0.0001	<0.0001	0.0142	<0.0001	<0.0001	0.0491	0.0003	<0.0001	0.0058	0.0351
F×S	0.7376	0.9108	0.4662	0.0021	0.1375	0.0709	0.0002	0.9549	0.7020	0.0929	0.8829	0.1980	0.0875
M×F×S	0.9991	0.6683	0.8772	<0.0001	0.1262	0.1401	0.0086	1.0000	0.0554	0.0571	0.1348	0.1183	0.1010

ns, *, **, *** not significant or significant at P<0.05, P<0.01, P<0.001. Data are analysed as an interaction using Least Squares means and means separated with Least Significant Differences of Tukey's.
[a] KCl = potassium chloride, OC = organic carbon (percentage of total soil mass), OC:N = ratio of organic carbon to nitrogen, N = total nitrogen, Total P = total phosphorus (mq/kg), Avail.P = available P (mg/kg), Ca^{2+}: calcium ; Mg^{2+} : magnesium ; K^{+} : potassium ; CEC : Cation Exchange Capacity; S : sum of bases (meq/100g) ;V :base saturation (%); N or Total N: Total nitrogen ; Season= Interrelationships between the end of season 1, the beginning and the end of season 2. Manure = Cow manure.

rate of cow manure was combined to 100 NPK rate, the yield was raised slightly and reached 21.65 t·ha^{-1}. Moreover, 150 NPK rate had a clearly beneficial effect as its combination with 40 CM raised the total yield from 12.52 to 20.10 t·ha^{-1} while that of 20 CM and 150 NPK and 30 CM and 150 NPK was respectively 14.95 and 19.31 t·ha^{-1}.

Relationship between season, cow manure and NPK concentration

The interrelationships among physicochemical and amendment parameters were showed in Table 7a and their mean square or degrees of freedom in Table 7b. Either the growing seasons or CM affected pH-KCl. NPK fertilizers affected OC: N ratio, the sum of bases, CEC, and saturation of bases. The interaction between CM and growing seasons affected pH-water, available P, organic carbon, the carbon-to-nitrogen ratio and calcium whereas that between CM and NPK affected organic carbon and OC: N ratio. The interrelationships among the growing seasons, CM and NPK affected total nitrogen, total P.

Evolution of physicochemical properties of the soil during the cultural and intercultural period

Tables 8, 9 and 10 showed physicochemical parameters values and Figures 1 to 3 based on the table's data show the evolution of these parameters against CM combine to NPK-fertilizer assessment during the two growing seasons studied. At the end of season 1 (Table 8), the average organic carbon (OC) level of the 16 treatments was 1.24±0.49%. In Figures 1 to 3, combinations of 0 t·ha^{-1} of cow manure and 50, 100 or 150 kg·ha^{-1} of NPK will be defined as NPK treatments and those of 20, 30 or 40 t·ha^{-1} of CM with 0 kg·ha^{-1} of NPK will be considered as cow manure treatments. The remainder combinations will stand for cow manure combined to NPK rates. Examination of Figure 1 showed for all studied parameters that combinations including 30 and 40 t·ha^{-1} of CM gave an overall level higher than the level reported in NPK treatments at the end of season 1. In Figure1A, magnesium level decreased at 50 kg·ha^{-1} of NPK rate, especially in 20 or 40 t·ha^{-1} of CM combined to NPK rates. The level stayed stable at 20 t·ha^{-1} of CM combined to NPK rates, at 100 or 150 kg·ha^{-1} of NPK rate but clearly increased in 40 t·ha^{-1} of CM combined to NPK rates. In 30 t·ha^{-1} of CM combined to NPK rates, Mg^{2+} level increased at 50 kg·ha^{-1} of NPK rate, starting with 1.92 meq/100 g, and decreased thereafter and reached 1.87 meq/100 g at 150 kg·ha^{-1} of NPK rate. Magnesium level increased in soil range from 1.34 to 2.29 times of the initial Magnesium reported before field trial in 2009. In Figure 1B, the S levels in 40 t·ha^{-1} of CM combined to NPK rates were higher than that identified in NPK treatments and increased with NPK rates. S levels in this combination were higher than S levels of 20 or 30 t·ha^{-1} of CM combined to NPK rates. In 30 t·ha^{-1} of cow manure combined to NPK rates, the S level increased at 50 kg·ha^{-1} of NPK rate, starting with

Table 7b. Mean square and degrees of freedom table for cow manure and/or NPK fertilizer and growing seasons effects on physicochemical parameters measured in 0-20cm depth on ferruginous soil.

Parameter[a]		pH water	pH KCl	OC(%)	N(%)	OC:N	Avail. P	Total P	Ca^{2+}	Mg^{2+}	K^{+}	S	CEC	V
Manure (M)	MS	1.800	0.348	1.540	0.003	57.436	228.008	10851.741	2.044	3.728	0.069	12.690	150.701	287.736
	Df	3.000	3.000	3.000	3.000	3.000	3.000	3.000	3.000	3.000	3.000	3.000	3.000	3.000
NPK (F)	MS	0.065	0.165	0.184	0.000	49.201	2.753	6045.759	0.089	1.026	0.022	1.862	67.506	808.608
	Df	3.000	3.000	3.000	3.000	3.000	3.000	3.000	3.000	3.000	3.000	3.000	3.000	3.000
Season (S)	MS	10.962	1.596	2.703	0.022	213.230	148.367	5129.915	13.000	2.473	0.841	31.173	515.865	1488.957
	Df	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000
Interactions														
M×F	MS	0.110	0.107	0.288	0.001	32.409	64.900	2551.111	0.018	0.687	0.007	0.699	7.924	175.172
	Df	9.000	9.000	9.000	9.000	9.000	9.000	9.000	9.000	9.000	9.000	9.000	9.000	9.000
M×S	MS	0.630	0.046	1.001	0.002	73.467	103.379	10051.914	1.418	0.377	0.072	3.233	18.928	321.067
	Df	6.000	6.000	6.000	6.000	6.000	6.000	6.000	6.000	6.000	6.000	6.000	6.000	6.000
F×S	MS	0.086	0.035	0.098	0.001	19.799	75.864	3851.002	0.024	0.102	0.023	0.097	8.299	256.200
	Df	6.000	6.000	6.000	6.000	6.000	6.000	6.000	6.000	6.000	6.000	6.000	6.000	6.000
M×F×S	MS	0.036	0.083	0.063	0.001	17.381	53.497	1646.117	0.014	0.291	0.025	0.365	5.242	204.995
	Df	18.000	18.000	18.000	18.000	18.000	18.000	18.000	18.000	18.000	18.000	18.000	18.000	18.000

[a]MS: Mean Square, Df: Degrees of freedom of the Table 7a data.

Table 8. Effects of cow manure and NPK fertilizers on phyco-chemical parameter of ferruginous soil at the end of Season 1.

Cow manure	0				20				30				40			
× NPK	0	50	100	150	0	50	100	150	0	50	100	150	0	50	100	150
	6.53	6.35	6.32	6.29	7.21	7.16	7.05	7.11	7.46	7.46	7.18	7.3	7.58	7.46	7.38	7.34
pHKCl	6.14	6.22	6.2	6.13	6.39	6.27	6.2	6.18	6.49	5.96	6.09	6.76	6.54	6.45	6.37	6.27
OC	0.48^{c}	0.59^{bc}	0.54^{bc}	0.54^{bc}	1.48^{ac}	1.37^{ac}	1.26^{ac}	1.15^{ac}	1.7^{ac}	2.1^{a}	1.27^{ac}	1.44^{ac}	1.93^{a}	1.76^{ab}	1.59^{ac}	1.43^{ac}
Mg^{2+}	0.06^{e}	0.07^{de}	0.09^{ce}	0.09^{ce}	0.07^{de}	0.08^{de}	0.10^{bce}	0.10^{bce}	0.10^{bce}	0.14^{ac}	0.06^{e}	0.14^{ac}	0.16^{a}	0.09^{bce}	0.13^{acd}	0.15^{ab}
Ca^{2+}	7.94^{ef}	7.97^{e}	6.18^{ef}	4.33^{f}	19.9^{a}	16.9^{ab}	13.2^{cd}	11.8^{cd}	17.9^{a}	12.1^{cd}	13.5^{bc}	12.4^{cd}	11.9^{cd}	18.6^{a}	12.6^{cd}	9.8^{de}
K^{+}	14.4^{g}	19^{f}	20.1^{ef}	22.9^{cf}	20.4^{ef}	21.1^{ef}	23.5^{bce}	26.5^{ac}	22.3^{def}	25.2^{acd}	24^{bce}	27.4^{ab}	23.9^{bce}	25.5^{acd}	26.6^{ac}	28.5^{a}
Total_N	97.2^{h}	143^{g}	158^{fg}	166^{ef}	112^{h}	158^{fg}	174^{df}	189^{bcd}	143^{g}	178^{df}	186^{bcde}	202^{ac}	174^{df}	181^{cde}	204^{ab}	220^{a}
CN	1.58^{b}	1.58^{b}	1.92^{ab}	1.99^{ab}	2.26^{ab}	2.12^{ab}	2.19^{ab}	2.26^{ab}	2.63^{ab}	2.7^{ab}	2.74^{ab}	2.75^{ab}	3.14^{ab}	3.2^{ab}	3.27^{a}	3.34^{a}
S	0.4^{b}	1.64^{ab}	1.73^{ab}	1.55^{ab}	2.47^{a}	1.5^{ab}	1.5^{ab}	1.55^{ab}	1.92^{ab}	2.24^{a}	1.85^{ab}	2.2^{a}	2.19^{a}	2.05^{ab}	2.24^{a}	2.56^{a}
AvailP	0.61	0.64	0.45	0.48	0.68	0.69	0.71	0.77	0.58	0.876	0.757	0.907	0.76	0.79	0.78	0.96
TotalP	2.59^{d}	3.86^{cd}	4.1^{bd}	4.01^{bd}	5.31^{abc}	4.32^{bd}	4.4^{bd}	4.58^{ad}	5.12^{abc}	5.52^{abc}	5.85^{abc}	5.65^{abc}	6.09^{abc}	6.05^{abc}	6.28^{ab}	6.85^{a}
CEC	9.8^{d}	11^{d}	11.4^{cd}	11.9^{cd}	14.1^{bc}	14.3^{bc}	15.1^{ab}	15.3^{ab}	16.5^{ab}	16.1^{ab}	17^{ab}	17.6^{a}	17.3^{a}	17.4^{a}	17.7^{a}	17.9^{a}
V	26.4^{g}	35.1^{ad}	36^{ac}	33.7^{cde}	37.6^{ab}	30.2^{f}	29.2^{fg}	29.9^{f}	31^{ef}	34.7^{bcd}	32.4^{df}	33.9^{cde}	35.2^{ad}	34.8^{bcd}	35.5^{ad}	38.3^{a}

[a-h]Means in a row without a common superscript letter differ ($P < 0.05$) as analysed by two-way ANOVA and the TUKEY test.

Table 9. Effects of cow manure and NPK fertilizers on phyco-chemical parameter of ferruginous soil at the beginning of Season 2.

Cow manure	**0**				**20**				**30**				**40**			
× NPK	**0**	**50**	**100**	**150**	**0**	**50**	**100**	**150**	**0**	**50**	**100**	**150**	**0**	**50**	**100**	**150**
pHwater	6.24	6.02	6.11	6.26	6.14	6.06	6.70	6.46	6.55	6.52	6.12	6.5	6.4	6.18	6.38	6.34
pHKCl	6.18	5.9	6.01	6.09	6.07	6.01	6.51	6.4	6.42	6.41	6.05	6.45	6.3	6.12	6.23	6.21
OC	0.624	0.84	0.84	1.11	0.93	0.682	0.88	0.63	1.17	1.01	0.60	0.69	0.76	0.67	0.81	0.77
Mg^{2+}	0.84^{d}	1.59^{bd}	1.99^{bc}	1.38^{bd}	1.73^{bc}	1.97^{bc}	1.61^{bd}	2.08^{ab}	1.45^{bd}	1.26^{cd}	1.83^{bc}	2.03^{bc}	2.05^{abc}	2^{bc}	2.15^{ab}	2.85^{a}
Ca^{2+}	1.49^{b}	1.57^{ab}	1.54^{ab}	1.59^{ab}	1.55^{ab}	1.52^{ab}	1.51^{ab}	1.73^{ab}	1.56^{ab}	1.57^{ab}	1.62^{ab}	1.65^{ab}	1.75^{a}	1.72^{ab}	1.62^{ab}	1.65^{ab}
K^{+}	0.55^{df}	0.61^{bcdf}	0.49^{f}	0.65^{ade}	0.66^{ad}	0.58^{cdf}	0.56^{df}	0.62^{bcde}	0.70^{ac}	0.53^{ef}	0.67^{ad}	0.72^{ab}	0.61^{bcdf}	0.59^{cdf}	0.76^{a}	0.62^{bcde}
Total_N	0.05^{b}	0.07^{ab}	0.07^{ab}	0.06^{ab}	0.07^{ab}	0.06^{ab}	0.07^{ab}	0.06^{ab}	0.08^{a}	0.07^{ab}	0.05^{ab}	0.07^{ab}	0.06^{ab}	0.06^{ab}	0.07^{a}	0.06^{ab}
CN	13.5	11.9	12.1	18.8	13.8	11	12.3	10	14.9	14.4	11.2	10.1	11.7	12	11.2	13.8
S	2.88^{e}	3.77^{bcd}	4.02^{bcd}	3.62^{ce}	3.94^{bcd}	4.07^{bcd}	3.68^{bce}	4.43^{ac}	3.7^{bce}	3.36^{de}	4.12^{bcd}	4.4^{ac}	4.4^{ac}	4.3^{ac}	4.53^{ab}	5.12^{a}
AvailP	27.9	30.4	22.1	26.1	25	24.6	31.5	23.2	18.8	26.8	28.6	23.5	35.1	22.4	32.9	25.7
TotalP	150^{ab}	130^{b}	132^{b}	154^{ab}	168^{ab}	187^{ab}	189^{ab}	170^{ab}	240^{ab}	245^{a}	217^{ab}	176^{ab}	201^{ab}	260^{a}	194^{ab}	203^{ab}
CEC	5^{c}	6^{bc}	12^{ac}	11^{ac}	6^{bc}	10^{ac}	11^{ac}	8^{ac}	8^{ac}	9^{ac}	9^{ac}	11^{ac}	13^{ac}	9^{ac}	15.3^{ab}	16.3^{a}
V	57.6	62.8	33.5	32.9	65.7	40.7	33.4	55.4	48.3	46.9	45.8	40	33.9	47.8	30.2	32

[a-g]Means in a row without a common superscript letter differ ($P < 0.05$) as analysed by two-way ANOVA and the TUKEY test.

Table 10. Effects of cow manure and NPK fertilizers on phyco-chemical parameter of ferruginous soil at the end of Season 2.

Cow manure	**0**				**20**				**30**				**40**			
× NPK	**0**	**50**	**100**	**150**	**0**	**50**	**100**	**150**	**0**	**50**	**100**	**150**	**0**	**50**	**100**	**150**
pHwater	6	6.19	6.04	6.08	6.15	6.18	6.21	6.19	6.38	6.41	6.12	6.13	6.43	6.15	6.39	6.06
pHKCl	5.81	5.78	5.81	5.92	5.77	5.79	5.99	6.01	6.14	5.76	6.04	5.89	6.27	6.01	6.18	5.89
OC	0.67	0.92	1.11	0.82	1.41	0.72	1.17	1.01	1.29	1.15	0.78	1.14	1.08	1.3	1.34	0.92
Mg^{2+}	0.89^{ef}	1.44^{bcde}	1.56^{ad}	1.32^{de}	0.77^{f}	1.52^{bcd}	1.40^{cde}	1.16^{df}	1.14^{df}	1.06^{df}	1.21^{df}	1.96^{ab}	1.94^{ac}	1.91^{ac}	1.56^{ad}	2.09^{a}
Ca^{2+}	1.48	1.5	1.52	1.58	1.52	1.55	1.54	1.67	1.55	1.53	1.47	1.62	1.61	1.62	1.57	1.57
K^{+}	0.54^{ab}	0.47^{ac}	0.51^{ac}	0.48^{ac}	0.38^{bc}	0.42^{ac}	0.42^{ac}	0.53^{ac}	0.60^{a}	0.45^{ac}	0.39^{bc}	0.42^{ac}	0.49^{ac}	0.43^{ac}	0.34^{c}	0.39^{bc}
Total_N	0.05^{b}	0.06^{ab}	0.06^{ab}	0.07^{ab}	0.06^{ab}	0.06^{ab}	0.06^{ab}	0.07^{a}	0.07^{ab}	0.06^{ab}	0.06^{ab}	0.07^{a}	0.07^{a}	0.07^{a}	0.07^{a}	0.06^{ab}
CN	12.9	16.1	17.6	12.7	21.8	12.2	18.4	14.2	20	17.7	12.1	15.6	15.1	18.2	18.3	15
S	2.91^{fg}	3.41^{bcef}	3.59^{ae}	3.38^{cef}	2.66^{g}	3.5^{aef}	3.36^{def}	3.36^{def}	3.29^{ef}	3.04^{eg}	3.08^{eg}	4^{ac}	4.04^{ab}	3.95^{acd}	3.48^{aef}	4.06^{a}
AvailP	14	16.1	18.5	27.9	21.2	18.7	23.4	20.2	36	32.4	24.3	26.6	33.6	28.5	20	19.9
TotalP	185^{ad}	189^{ad}	162^{bcd}	173^{ad}	156^{bcd}	215^{ad}	264^{a}	228^{ac}	164^{bcd}	250^{ab}	151^{cd}	145^{cd}	129^{d}	134^{cd}	156^{bcd}	183^{ad}
CEC	4^{b}	6^{ab}	10^{ab}	8.87^{ab}	7^{ab}	8^{ab}	9^{ab}	11^{ab}	8^{ab}	10^{ab}	9^{ab}	9^{ab}	8^{ab}	10^{ab}	11^{ab}	13^{a}
V	72.8^{a}	56.9^{ab}	35.9^{ab}	37.6^{ab}	38^{ab}	43.7^{ab}	37.4^{ab}	30.6^{b}	41.1^{ab}	31.8^{ab}	34.2^{ab}	44.4^{ab}	50.5^{ab}	39.5^{ab}	31.6^{b}	31.2^{b}

[a-g]Means in a row without a common superscript letter differ ($P < 0.05$) as analysed by two-way ANOVA and the TUKEY test.

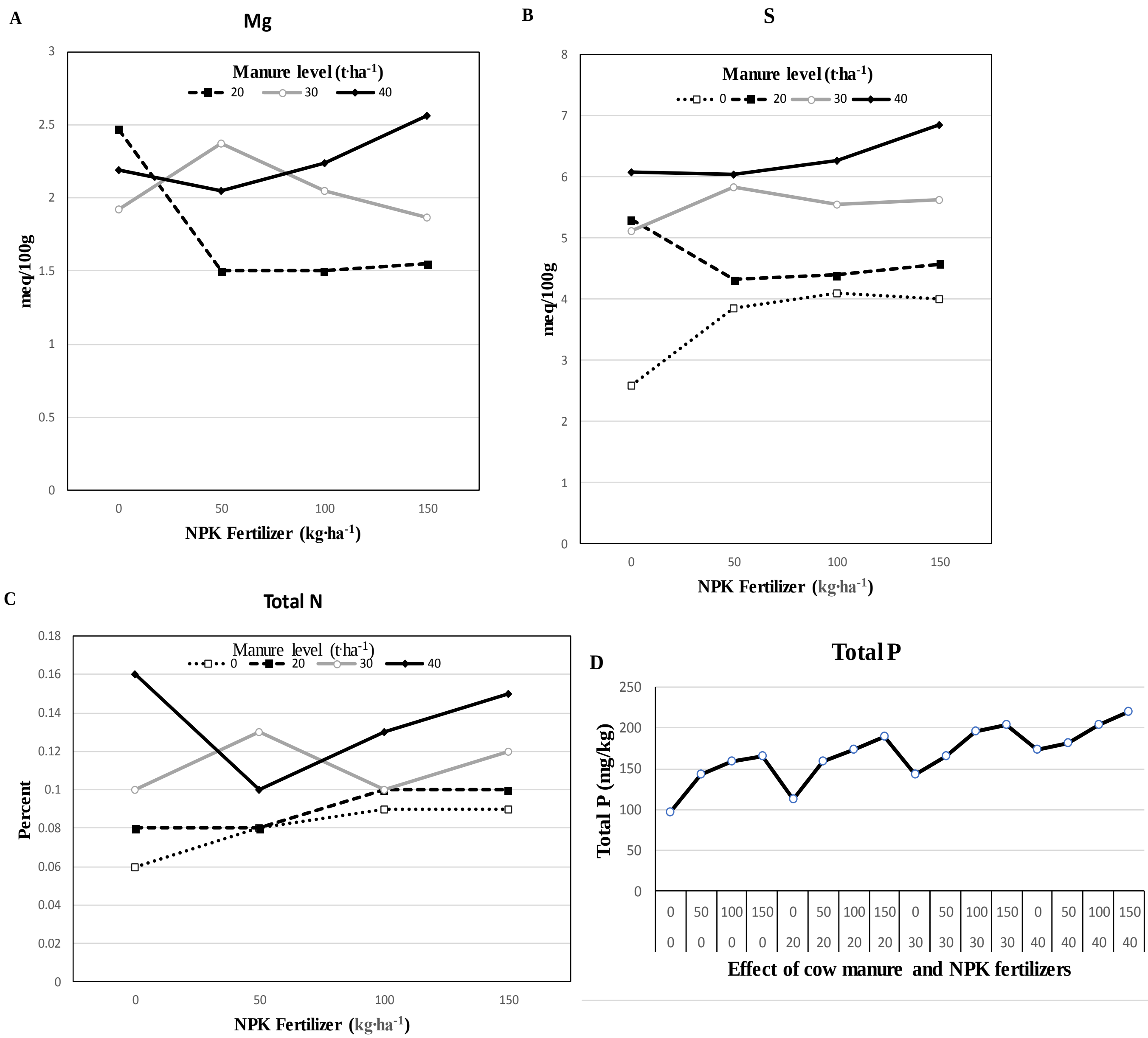

Figure 1. (A) Magnesium, (B) Sum of bases, (C) total nitrogen, (D) total phosphorus at the end of Season 1.

5.12 meq/100 g, decreased later and became almost constant from 100 to 150 kg·ha^{-1} of NPK rate. In 20 t·ha^{-1} of cm combined to NPK rates, S level decreased with the increase rate of NPK. We should report here that, the S level of 20 t·ha^{-1} of CM treatment was higher than that of 20 t·ha^{-1} of CM combined to NPK rates while in NPK treatments, the lowest S was reported from the control (2.59 meq/100 g).

In Figure1C, with starting value around 0.16% (40 t·ha^{-1} of CM treatment), the total N content decreased for the treatment with a large number of organic restitutions (40 t·ha^{-1} of CM combined to 50 kg·ha^{-1} of NPK). It increased thereafter in the other treatments and reached a higher value of 0.15%. The NPK treatments evolved in the lower part of the figure, reaching a value near 0.09% with 150 kg·ha^{-1} of NPK treatment. Interestingly, in 20 t·ha^{-1} of CM combined to NPK rates, the total N increased with NPK rates and stabilized for 100 and 150 kg·ha^{-1} of NPK rate. Total N level of 30 t·ha^{-1} of CM combined to NPK rates decreased at 100 kg·ha^{-1} of NPK rate and increased, later on, reaching 0.12% with 150 kg·ha^{-1} of NPK rate. The levels of total N in 30 t·ha^{-1} of CM treatment and 30 t·ha^{-1} of CM combined to 150 kg·ha^{-1} of NPK were similar. Total N level increased in soil range from 1.14 to 2.29 times of the initial total N reported before field trial in 2009. Total P (Figure1D) levels of the soil in 40 t·ha^{-1} of CM combined

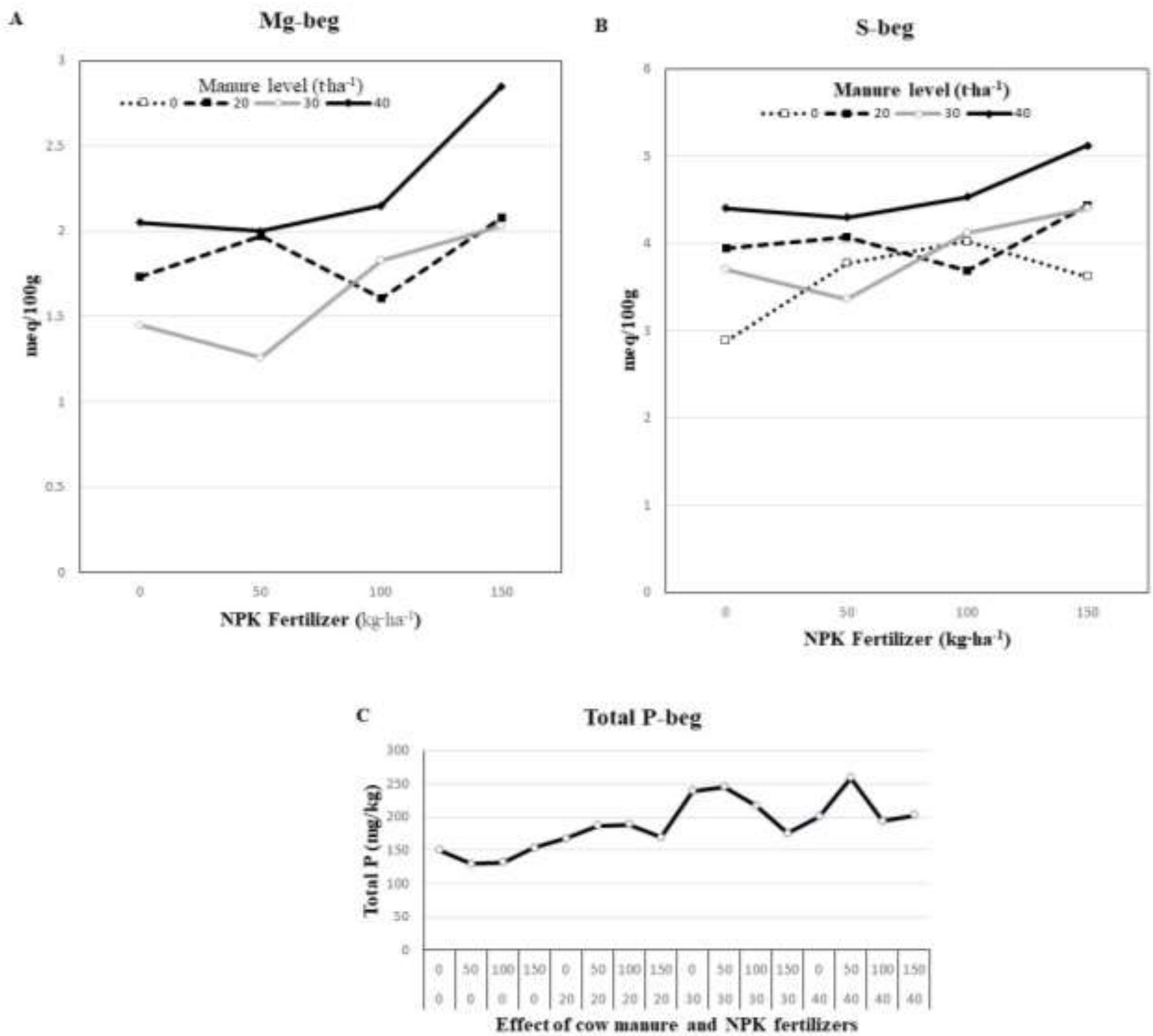

Figure 2. (A) Magnesium, (B) Sum of bases, (C) total phosphorus at the beginning of Season 2.

to 50, 100 or 150 NPK rates were higher than the levels identified in NPK treatments or cow manure treatments. Interestingly, total P levels always drop in cow manure treatments compared to the remainder treatment. In addition, the levels of this parameter increased with NPK rates. Total P level increased in soil ranged from 2.35 to 4.60 times of the initial total P reported before field trial in 2009. At the beginning of Season 2 (Table 9), total nitrogen average value of 0.06±0.01 is slightly lower than that reported before field trial (0.07%) and close to that of the control at the end of season 1 (0.06%). OC (%) average value of 0.81±0.17 was lower than that of the end of season 1 and decreased by 0.21% based on the value got before field trial. OC: N ratio average level of 12.67±2.19 was lower than OC: N levels of 20 CM and 0 NPK, 20 CM and 50 NPK, 30 CM and 17.89 NPK, 30 CM and 100 NPK or 40 CM and 50 NPK treatments at the end of season 1. Available P, the average level of 26.53±4.33 mg/kg, reported here was 1.53 times higher than that of the beginning of season 1 (17.3 mg/kg).

In Figure 2, the levels of magnesium and S in 40 t·ha^{-1} of CM combined to NPK rates were higher than those of corresponding parameters in NPK treatments. In Figure 2A, the highest amount of magnesium was reported in 40 t·ha^{-1} of CM and 150 kg·ha^{-1} of NPK. This value was 1.29 times higher than that of 40 t·ha^{-1} of CM treatment. The level of magnesium also dropped in 30 t·ha^{-1} of CM and 50 kg·ha^{-1} of NPK. It increased latter with the increase rate of NPK from 50 to 150 kg·ha^{-1}. For 20 t·ha^{-1} of CM combined to NPK rates, the level of magnesium increased alongside NPK rates but a drop was reported at 100 kg·ha^{-1} NPK rate. Magnesium level increased in soil ranged from 1.13 to 2.55 times of the initial Magnesium reported before field trial in 2009. For the S parameter (Figure 2B), the amount reported in 40 t·ha^{-1} of CM combined to NPK rates was above both that of NPK treatments and 20 or 30 t·ha^{-1} of CM combined to NPK rates. The highest amount reached is 5.12 meq/100 g. Strikingly, starting at 50 kg·ha^{-1} of NPK rate, the amount of S in 30 or 40 t·ha^{-1} of CM combined with NPK rates increased with the increase of NPK rate. Again, starting at the same rate (50 kg·ha^{-1} of NPK), the amount of S in 30 or 40 t·ha^{-1} of CM combined to 150 kg·ha^{-1} of NPK increased while that of 20 t·ha^{-1} of CM combined to 100 kg·ha^{-1} of NPK decreased and increased later at 150 kg·ha^{-1} of NPK rate. We noticed that, the levels of S in 30 or 40 t·ha^{-1} of CM treatments were higher than those of 30 or 40 t·ha^{-1} of CM combined to 50 kg·ha^{-1} of NPK , but it was lower than the level reported in 30 or 40 t·ha^{-1} of CM combined to 100 or 150 kg·ha^{-1} of NPK. In NPK treatments, the level of S in the control was the lowest S value identified (2.88 meq/100 g). The S level increased when NPK treatments moved from 50 to 100 kg·ha^{-1} but it decreased to 150 kg·ha^{-1} of NPK treatment. Total P level increased in soil ranged from 2.72 to 5.43 times of the initial total P reported before field trial in 2009 (Table 9).

In Figure 2C, total P level of 20 t·ha^{-1} of CM combined to NPK rates decreased, starting from 187.39 mg/kg. A total P level of 30 t·ha^{-1} of CM combined to NPK rates decreased, starting from 245.32 mg/kg; while that of 40 t·ha^{-1} of CM combined to NPK rates decreased and increased, later on, starting from 259.55 mg/kg. A total P level of NPK treatments increased, starting from 130.02 mg/kg. Surprisingly, total P levels of all treatments involving 100 or 150 kg·ha^{-1} of NPK combined to 20, 30 or 40 t·ha^{-1} of CM showed a decrease in the level of phosphorus compared to the remainder treatments. At the end of Season 2 (Table 10), OC: N average level of 16.12±2.89 was higher than that reported at the beginning of season 2 (12.67). OC (%) average value of 1.05±0.23 (Table 10) was higher than that noticed at the beginning of the season 2 (Table 8). It increased only by 2.94% based on OC level before field trial. The level pH-water of 30 t·ha^{-1} of CM combined to NPK rates decreased with the increase rate of NPK while that of 20 t·ha^{-1} of CM combined to NPK rates increased with the increase rate of NPK. The pH-water of 30 t·ha^{-1} of CM was 1.05 times higher than that of 30 t·ha^{-1} of CM combined to 100 kg·ha^{-1} of NPK. The highest pH-water value was reached in 40 t·ha^{-1} of CM treatment (6.43) and the lowest was reported in 40 t·ha^{-1} of CM combined to 150 kg·ha^{-1} of NPK (6.06). Obviously, relative to pH value reported before the field trial, it decreased by 4.60. In NPK treatments, pH-water level decreased with the increase rate of NPK.

In Figure 3A, magnesium level increased in 30 t·ha^{-1} of CM combined to NPK rates with the increase of NPK rate and reached a higher value of 1.96 meq/100 g. The magnesium level of 30 t·ha^{-1} of CM treatment was higher than that of 30 t·ha^{-1} of CM combined to 50 kg·ha^{-1} of NPK but lower than that 30 t·ha^{-1} of CM combined to 100 or 150 kg·ha^{-1} of NPK. The magnesium level of 20 t·ha^{-1} of CM treatment was lower than that of 20 t·ha^{-1} of CM combined to NPK rates. Here, magnesium level decreased with the increase of NPK rates. Interestingly, the magnesium level in 40 t·ha^{-1} of CM combined to NPK rates decreased with the increased NPK rates except for 40 t.ha^{-1} of CM combined to 150 kg·ha^{-1} of NPK. Surprisingly, 100 kg·ha^{-1} of NPK treatment and 40 t·ha^{-1} of CM combined to 100 kg·ha^{-1} of NPK had the same level of magnesium. Magnesium level increased in soil ranged from 1.02 to 1.87 times of the initial Magnesium reported before field trial in 2009, except for 20 t·ha^{-1} of CM treatment and 30 t·ha^{-1} of CM combined to 50 kg·ha^{-1} of NPK. In Fig.3B, S level of 20 t·ha^{-1} of CM treatment was than that of 20 t·ha^{-1} of CM combined to NPK rates. Moreover, the S of the control was lower than that identified in NPK treatments. Here, S appears to increase with the increase of NPK rates but a drop occurred in 150 kg·ha^{-1} of NPK treatment. At 150 kg·ha^{-1} of NPK rate, the S of 30 and 40 t·ha^{-1} of CM combined to NPK rates increased, starting respectively with 3.08 and 3.48 meq/100 g. It was also obvious that the S level of 40 t·ha^{-1} of CM combined to NPK rates was above that of 20 or 30 t·ha^{-1} of CM combined to NPK rates. This trend was extending to NPK treatments, except for 100 kg·ha^{-1} of NPK treatment.

In Figure 3C, total N level in both 30 t·ha^{-1} of CM combined to NPK rates increased with the increase of NPK rate. In NPK treatments, total nitrogen level increased, starting with the level reported from the control. It had the same value in 50 and 100 kg·ha^{-1} of NPK treatments, and increased in 150 kg·ha^{-1} of NPK treatment. Again, the general trend noticed, was that total N level between 50 and 100 kg·ha^{-1} of NPK rate is always constant. In addition, considering 30 t·ha^{-1} of CM treatment and 30 t·ha^{-1} of CM combined to NPK, when NPK was brought to the field, total nitrogen level dropped from 0.07 to 0.06%, became constant and increased back to 0.07%. Interestingly, total nitrogen level of 20 t·ha^{-1} of CM treatment was similar to that of 20 t·ha^{-1} of CM combined with 50 or 100 kg·ha^{-1} of NPK.

In Figure 3D, total phosphorus level in 40 t·ha^{-1} of CM combined to NPK rates increased with the increased of NPK rates while it decreased in 30 t·ha^{-1} of CM combined to NPK rates with the increase of NPK rates. In addition, the aforementioned decrease of total P levels in treatments involving 100 or 150 kg·ha^{-1} of NPK combined to 20 or 30 t·ha^{-1} of CM, reported at the beginning of season 2, were observed as well. Nonetheless, a slight increase of total P levels was reported in 100 or 150 NPK combined to 40 t·ha^{-1} of CM. The total P level of 20 t·ha^{-1} of CM combined to NPK rates reached its highest value of 263.78 mg/kg at 100 kg·ha^{-1} of NPK rate, which was 1.69 times higher than that of 20 t·ha^{-1} of cow manure treatment. Besides, the highest total P level of 249.59

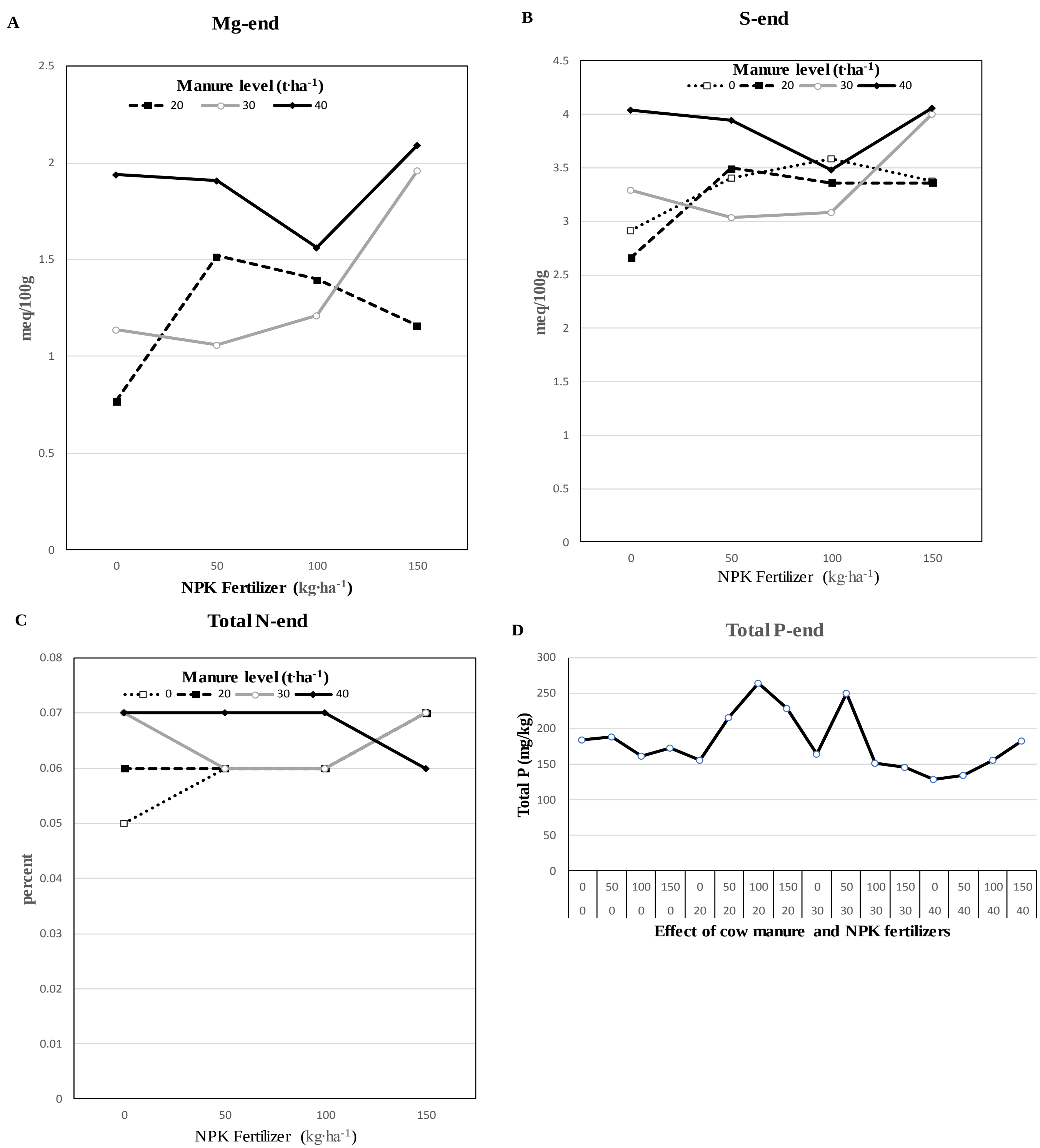

Figure 3. (A) Magnesium, (B) Sum of bases, (C) total nitrogen, (D) total phosphorus at the end of Season 2.

mg/kg reported with 30 t·ha^{-1} of CM combined to 50 kg·ha^{-1} of NPK was 1.72 times higher than the total P level of 30 t·ha^{-1} of CM combined to 150 kg·ha^{-1} of NPK but 1.52 times higher than that of 30 t·ha^{-1} of CM treatment. The total P amongst NPK treatments appears to decrease with the increasing rates of NPK. Total P level increased in soil ranged from 2.69 to 5.52 times of the initial total P identified before field trial in 2009. Taking together, all data suggesting a variable contribution of total P, magnesium and nitrogen during the growing seasons that was more prominent than the other chemicals parameters tested.

DISCUSSION

Effect of amendment on sesame yield and growth parameters

In amended context and unamended conditions, NPK fertilizers at some levels appear to affect all type of yield reported. These findings were explained because NPK at a rate of 50 or 100 kg·ha^{-1} gave a total yield higher than that of 20, 30 or 40 t·ha^{-1} of cow manure. Therefore, NPK noticeably influenced the survey sesame species only at 100 or 50 kg·ha^{-1}. From our results, it emerged for all treatments including 0 , 20 or 40 t·ha^{-1} of cow manure combined to 0, 50 or 100 kg·ha^{-1} of NPK, that the yield was above 16.55 t·ha^{-1}, but with 40 t·ha^{-1} of cow manure and 150 kg·ha^{-1} NPK, the yield was 14.72 t·ha^{-1}. Therefore, the yield appears to be higher with NPK fertilizers than with cow manure application. The higher performance reported with sesame in NPK over cow manure shows the ease of dissolution of nutrients in the inorganic fertilizer being in a more soluble form. However, NPK at a rate of 150 kg·ha^{-1} has both beneficial and detrimental effect on sesame yield. Indeed, the total yield level rose up from 16.55 to 19.70 or 22.68 t·ha^{-1} when 150 kg·ha^{-1} of NPK was combined either to 20 or 30 t·ha^{-1} of cow manure. In contrast, the yield dropped when it was combined with 40 t·ha^{-1} of cow manure (14.72 t·ha^{-1}).

In unamended case, cow manure gave a yield (20.40 t·ha^{-1} for 20 and 0 kg·ha^{-1} of NPK) higher than that of the whole NPK rate (50, 100 and 150 kg·ha^{-1}). These results are in agreement with Natsheh and Mousa (2014)'s observations showing that plants produced by inorganic fertilizers showed relatively lower yield compared to organic materials. Industrial fertilizers do not own good characteristics of aggregating the soil particles (Seyed, 2014). This may explain the relatively lower yield obtained with 100 kg·ha^{-1} of NPK in Season 1 compared to that of 20 t·ha^{-1} and 100 kg·ha^{-1} of NPK of Season 2.

Our results aligned with Xu et al. (2005)'s findings highlighted that vegetables grown with organic fertilizers resulted in a higher total yield than those grown with chemical fertilizers. Liu et al. (2016) reported that crop yields improved after addition of organic fertilizers. Nevertheless, the slight yield increase in control plot during the second year may be due to a seasonal effect.

Furthermore, the significant effect of cow manure on leaves yield combined with that of NPK fertilizers at 100 kg·ha^{-1} appears to have a sustainable effect on leaves and stems yields and thereby improved total yield during the second growing season. Indeed, the treatment 20 t·ha^{-1} and 100 kg·ha^{-1} of NPK that produced the highest total yield reported in season 2 were consistent with this trend. In this report, for the overall growing seasons, yields leaves and total yield reported for 0 t·ha^{-1} of cow manure and 100 kg·ha^{-1} of NPK were higher when compared with those from 20 t·ha^{-1} of cow manure and 100 kg·ha^{-1} of NPK. In particular, it may be due to the lower availability of nutrients from organic sources in the second growing season.

As cow manure and NPK fertilizers significantly affected organic carbon or NPK fertilizers as well as cow manure affected only leaves yield we can state that fertilizers application was a necessary condition for good sesame yield. On the other hand, growing seasons affected the total yield, leaves yield and stems yield suggesting the relevancy of this parameter for sesame growth. In addition, cow manure can be used to sustain sesame yield, reduce the amount of toxic compounds produced by conventional fertilizers, hence, improving soil fertility or the quality of leafy vegetables produced and supplying more leaves for food diet. In the meantime, we can also choose an adequate type of fertilization as cow manure and NPK significantly affected both organic carbon and carbon-to-nitrogen ratio. The height and number of branches were lower in season 1 than in Season 2 suggesting the progressive release of nutrients on time either from NPK or from cow manure.

Regarding the data before experiment, we concluded, for 40 t·ha^{-1} of cow manure and 50 kg·ha^{-1} of NPK or 0 t·ha^{-1} of cow manure and 100 kg·ha^{-1} of NPK treatments of the end of Season 1 that fewer P stocks were formed and more P was available. Again, total N and S of 40 t·ha^{-1} of cow manure combined with 50 kg·ha^{-1} of NPK that were higher than those of 0 t·ha^{-1} of cow manure and 100 kg.ha^{-1} of NPK appear to be conducible for more leaves yield. The increased levels of Mg^{2+} and K^+ show to some extent the contribution of cow manure and NPK to our ferruginous soil.

Climatic effects on sesame yield

Climatic components may affect yields data of the two growing seasons (rainfall, solar radiation or temperature). Indeed, Akinyele and Osekita (2006) found that the yield of any crop depends on existing climatic and ecological factors. In our report, the yields of season 1 were subjected to 97.10, 148.00 and 216.00 mm/day of rainfall as well as 29.10 to 32.20°C maximal temperature or 22.30 to 23.00°C minimal temperature. In contrast, during season 2, yields resulted from 80.20 mm/day, 99.40 and 273.00 mm/day of rainfall with 30.20 to 32.90°C maximal temperature as well as 22.40 to 23.80°C minimal temperature. Sesame optimum temperature average is 20°C (Nyabyenda, 2005). Also, Season 2 (2010) monthly rainfall from July to November watering the soil increased from 1.59 to 3.12 times of the initial monthly rainfall reported from the same period in 2009. Therefore, we presumed rainfall and temperature variations result in a difference of yields and nutrient availability. Temperature and soil moisture influence the rate of decomposition and nutrient release (Mohammed et al., 2013, 2014). Besides, Cissé et al. (2016) found a significant correlation between the rainfall onset date and the start of the growing season on ferruginous tropical soil.

Soil nutrients fluctuation on ferruginous soil regarding the survey treatments

We found that pH-water and pH-KCl are affected by cow manure. Both parameters declined in some unamended plots. The higher soil pH level in 20, 30 or 40 t.ha^{-1} combined with 0 kg.ha^{-1} of NPK fertilizers was consistent with Braos et al. (2015)'s findings. In fact, these authors reported an increase in soil pH after applying 20 t.kg^{-1} of cattle manure as organic fertilizers. This increase of pH level was due to the presence of calcium, bicarbonate anion (HCO_3^-) buffer effect and organic acid anions in the cattle manure (Braos et al., 2015).

We also reported at the end of season 2 a ratio OC: N of 16.12 falling between 15 and 25. It occurred when cow manure was add to the soil (Hazelton and Murphy, 2007) and indicating a slow in the decomposition process of soil organic matter according to Baize (2000). Our results showed that soil carbon levels could be increased reasonably easily, remained at elevated levels with inputs but reverted back to the sustainable base level in time as its level was 1.02, 1.24, 0.81 and 1.05% from the beginning to the end of the experiment. Some parts of total N content of the soil at the end of season 2 clearly depended on NPK fertilizers. The other parts depended on 20 or 30 t.ha^{-1} of cow manure combined to 50 to 150 kg.ha^{-1} of NPK. We assuming here that the low contribution of manure treatments to N content involved a mineralization speed processes. Indeed, most N in manure is usually present in organic forms and need to be mineralized to inorganic forms ($NH4^+$ and NO_3^-) before being available for plant uptake (Gai et al., 2016). In addition, we showed that NPK fertilizers affected the sum of the bases, CEC and base saturation. The organic carbon content affected by cow manure meaning that cow manure was relevant to soil organic carbon improvement.

The variations of cations levels reported may be due to the interference of some nutrients with the uptake of a nutrient that would normally be inadequate supply. Lower content of magnesium is typically found in sandy soils (Gransee and Führs, 2013). Diouf et al. (1999) reported that traditional leafy vegetables were rich in nutrients and minerals and exhausted the soil by taking away P, K^+ and other minerals such as Mg^{2+}. These observations aligned with the loss of Mg^{2+} occurred in our survey at the end of season 2. The decrease of Mg^{2+} reported in 20 t.ha^{-1} cow manure combined to NPK rates at the end of season 2 illustrated an unbalance fertilization or antagonism between Mg^{2+}, K^+, and other soils cations. It rose up the relevancy of Mg^{2+} for sesame photosynthesis or its exhaustion by leaching and crop removal. Besides, several plant metabolic processes were affected by magnesium. From the beginning to the end of season 2, total P level was higher than available P level. Total P stocks of the end of Season 2 decreased in 30 t.ha^{-1} cow manure combined to NPK rates or 100 kg.ha^{-1} of NPK treatments; meaning that parts of total P stocks from these treatments were in available forms. It emerged that P uptake occurred only in anionic forms as H_2PO4^- and $HPO4^{2-}$, and strong P fixation was commonly observed through sorption or precipitation reactions in soils. Therefore, the fixation of P compounds by iron, aluminium oxides or soil organic matter and their degree of insolubility were responsible for the low availability of its assimilable forms for crops (Behera et al., 2014). Therefore, the remainder P accumulated in the soil and the excess amounts of P reported at the end of Season 2 for some treatments were understandable. Nonetheless, Hazelton and Murphy (2007) reported that legumes cannot grow and keep nitrogen without an appropriate level of phosphorus. Further, the formation of complexes between calcium and phosphate may restrict diffusion and adsorption of phosphate by plants. The difference in P levels, from the end of Season 1 to 2, may be due to P surface runoff, erosion and leaching. In addition, we found from our results that total phosphorus decreased in NPK treatments while increased with 40 t.ha^{-1} combined to NPK rates. It means that the presence of cow manure probably maintained an adequate supply of organic matter in soil that improved soil physical and chemical condition and led to the increase of phosphorus and CEC in 40 t.ha^{-1} combined to NPK rates. This trends probably also explained the increase of Mg^{2+} level in 30 t.ha^{-1} of cow manure combined to NPK rates at the end of Season 2. However, the small increase in Mg^{2+} contents of 40 t.ha^{-1} of cow manure combined to NPK rates was possibly due to a low content of this nutrient in cattle manure. Mantovani et al. (2017) have found a low content of Mg^{2+} in cattle manure. Ewulo (2005) reported that addition of cattle manure to soil leads to increase in soil CEC, phosphorus, and magnesium. CEC can be also affected by the positively charged ions, K^+ and $NH4^+$, provided by NPK fertilizers. In addition, available magnesium in soil increased as result of cations antagonism that exists between calcium, magnesium and potassium (Senbayram et al., 2015). Considering 0 t.ha^{-1} of cow manure combined to 100 kg.ha^{-1} of NPK and 20 t.ha^{-1} of cow manure combined to 100 kg.ha^{-1} of NPK that gave a higher yield, respectively, during Season 1 and 2, we found at the end of season 2 that total N levels were alike. For 0 t.ha^{-1} of cow manure combined to 100 kg.ha^{-1} of NPK, total P decreased while magnesium and S increased. We reported for 20 t.ha^{-1} of cow manure combined to 100 kg.ha^{-1} of NPK that total P level increased while S level decreased. Therefore, magnesium, S and total phosphorus appear to be significant for improving sesame yields. Again, cow manure and NPK fertilizers were not affecting the same parameters, except for organic carbon.

CONCLUSIONS AND PERSPECTIVES

This work shows that organic and inorganic fertilizers are relevant for sesame growth on the ferruginous soil. Further, in unamended conditions, 20 t·ha^{-1} of cow manure combined to 100 kg·ha^{-1} of NPK, improving sesame total yield and leaves, pointing out its relevancy for sustainable yield. It is therefore, recommended that 20 t·ha^{-1} of cow manure and 100 kg·ha^{-1} of NPK be used in the cultivation of *S. radiatum* and particular attention should be paid to magnesium, sum of bases and total phosphorus level of the soil for sustainable production. It will be also valuable to enhance our existing knowledge on this leafy vegetable via a genetic characterization of its diversity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Impact of companion cropping on incidence and severity of maize ear rots and mycotoxins in Western Kenya

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Mycotoxins are harmful to health and mainly arise from ear rots, affecting maize in the field. This work analysed the effect of the cropping system on ear rot and final effect on mycotoxins from four sub-counties (districts) of western Kenya, Butere, Kisumu, Siaya and Vihiga, where plots comprising maize planted either as pure stand or in mixture with legumes, predominantly common bean treated as "Maize Monocrop" (MM), were used as control for those of climate-smart push-pull strategy treated as "Push-Pull" (PP). Symptomatic and asymptomatic maize ear samples were analysed for total aflatoxin (AF), total fumonisins (FB), deoxynivalenol (DON) and zearalenone (ZEA) using Enzyme-Linked Immunosorbent assay (ELISA). Cropping system had very high significant effect on ear rot incidence and severity. In general, low incidence was observed in PP (7.3 %) than MM (20.8 %). Similar trend was also observed on ear rot severity in PP and MM as follows: diplodia (1.15 and 1.85), gibberella (0.62 and 0.84), aspergillus (0.09 and 0.25), fusarium (0.19 and 0.68) and penicilium (0.03 and 0.05). A high proportion of ZEA (100%), AF (93.3%), DON (80.0%) and FB (65.9%) were observed in symptomatic samples than in ZEA (90.3%), DON (51.6%), FB (38.7%) and AF (3.2%) in asymptomatic samples. Low ear rot incidence and severity was more in PP than MM, and proportion of mycotoxins on asymptomatic ears; suggesting the potential of cropping system in managing ear rots and ultimately limiting mycotoxins. Thus the study highlighted the need to adopt cropping systems to deal with mycotoxins, and also recommends surveillance and awareness on emerging mycotoxins: ZEA and DON.

Key words: Push-pull, maize monocrop, aflatoxins, fumonisins, deoxynivalenol, zearalenone.

INTRODUCTION

Maize ear rots are fungal infections with worldwide distribution and presence in all agro-ecologies where maize is grown (Dragich and Nelson, 2014). Key fungal genera prominent for maize ear rot infections in sub-

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Saharan Africa include *Aspergillus*, *Fusarium*, *Sternocarpella* and *Penicilium* (Kapindu et al., 1999), causing estimated yield losses ranging between 10 and 30% (Kapindu et al., 1999; Ajanga and Hillocks, 2000; Bigirwa et al., 2007). Ravages caused by the ear rots are aggravated by mycotoxins, which are increasingly becoming the main sources of grain losses globally. Four agriculturally important mycotoxins with high occurrence are fumonisins, zearalenone, deoxynivalenol and aflatoxins (Gxasheka et al., 2015). These mycotoxins pose high risks to human and animal health (Zain, 2011), and have led to stringent regulation of their levels in food and feed in global grain trade (Otsuki et al., 2001).

A number of pre- and post-harvest measures have been advanced to manage ear rots and mycotoxin contamination in grain crops (Hell et al., 2008), with sorting as a post-harvest measure being key. Studies have directly linked mycotoxin reduction to removal of fungal ear or kernel rot (Balconi et al., 2014), making sorting a primary tool for management of mycotoxins and quality enhancement of the harvested crop (Wild et al., 2016). Moreover, uses of normal grain cleaners, largely in developed economies, have resulted in 50-60 % reduction of fumonisins and aflatoxins in some countries (Malone et al., 1998; Pacin and Resnik, 2012). Further improvement on grain sorting has also been made through development of high-capacity electronic optical sorters which target kernel discoloration and mycotoxin fluorescence (Pearson et al., 2010). Similar results have been obtained in Africa, where for instance, reports indicate 40% reduction of aflatoxin concentration through removal of moldy, damaged and broken grains in Benin (Fandohan et al., 2005). In addition, reports indicate 20% reduction of fumonisins in Tanzania, Kenya and South Africa as a result of sorting (Kimanya et al., 2009; van der Westhuizen et al., 2010; Mutiga et al., 2014). However, owing to the fact that 80 % of arable land (Wiggins, 2009) and 75% of total maize production (Nyoro et al., 1999) are from smallholders farmers, mycotoxin recycling and high exposure to consumers are preeminently promoted by alternative use of infected ears by fungal rot (Bigirwa et al., 2007; Mukanga et al., 2011). Since ear rot attacks begin in the field, pre-harvest measures can ensure achievement of good yields and grain quality in terms of mycotoxin reduction (Munkvold, 2003).

Generally, factors including fungal taxon, humidity, rainfall, insect damage, drought, irrigation and maize germplasm influence incidence of maize ear rots in the field (Parsons, 2008). Gibberella and diplodia ear rots, caused by *Fusarium graminearum* and *Stenocarpella maydis*, respectively, are encouraged by high rainfall, susceptible crop genotypes, continuous maize cultivation and poor crop residue management (Marasas, 2001). The recurrence of fumonisin and aflatoxin contamination in tropical regions, make fusarium and aspergillus ear rots, caused mainly by *Fusarium verticillioides and Aspergillus flavus*, respectively, of high concern; although diplodia ear rot exceed them on incidence in several studies in maize growing regions (Bigirwa *et al.,* 2007; Mukanga *et al.,* 2010). Among the favorable factors for *Fusarium* and *Aspergillus* ear rots are water stress, insect damage and nutritional status of soil (Marasas et al., 2001). Empirically, strong correlation of ear rots with insect attack, and correlation of silk-cut symptom with incidence of immature thrips population has been reported (Parsons, 2008; Ajanga and Hillocks, 2000). Thus reduction in insect damage has resulted in reduced ear rots and mycotoxin attacks. These measures are aimed to control damage to crops by insect pests, thus contributing to the management of ear rots and mycotoxin attacks on harvested crop (Munkvold et al., 1997).

Push-push technology, a novel companion cropping system where maize is intercropped with insect repellent forage legumes in the genus *Desmodium* and with grasses such as *Brachiaria* planted around this intercrop effectively controls stemborers, the key pests of cereals in eastern Africa (Khan et al., 2014; Midega et al., 2015a,b). The technology, which is practiced by over 130,000 smallholder farmers in eastern Africa to date, has the potential to contribute to management of ear rots and mycotoxin contamination in maize in the region. This study analyze the influence of push-pull technology on incidence and severity of maize ear rots in maize and quantify the level, incidence and range of mycotoxins on fungal infected (symptomatic) and clean (asymptomatic) maize ears.

MATERIALS AND METHODS

Study site

The study was conducted in Butere (0° 09' to 0° 20' S, 34° 29' to 34° 33' E), Vihiga (0° to 0° 15' S, 34° 30' to 35° 0' E), Kisumu (0° 15' to 0° 25' S, 34° 55' to 34° 67' E) and Siaya (0° 26' to 0° 18' S, 33° 58' to 34° 33' E) sub-counties (districts) of western Kenya, where push-pull technology has been widely disseminated and has been practiced by smallholder farmers since the year 2000 (Figure 1) (Khan et al., 2011). The study sites are characterized by a bimodal rainfall pattern and forms part of the larger grain basket of Kenya. The region has the highest concentration of smallholder farmers who grow maize largely in mixed stands with legumes and in combination with livestock (Khan et al., 2011). Occurrence of ear rots is one of the key constraints affecting growing and utilization of maize in the region (Ajanga and Hillocks, 2000). Other serious constraints also include insect pests, principally cereal stemborers, striga weeds and poor soil fertility (Midega et al., 2015b). Studies have shown a strong and positive correlation of ear rots (r=0.87) with insect damage (Ajanga and Hillocks, 2000). The current study was conducted in farmers' fields during the short (September to December) rainy season of 2014 and the long rainy season (March to August) of 2015, with treatments comprising maize grown either in push-pull or in sole stands (monocrop). In both plots, maize was planted at inter and intra-row spacing of 75 and 30 cm, respectively. The push-pull treatment had maize intercropped with greenleaf desmodium (*Desmodium intortum* (Mill.) Urb.), with *Brachiaria* cv Mulato II grown as a border crop around this intercrop at a spacing of 50 cm within and 50 cm between rows. Farmers in the sample districts planted their local maize varieties, ‘Nyamula’ and ‘Jowi’

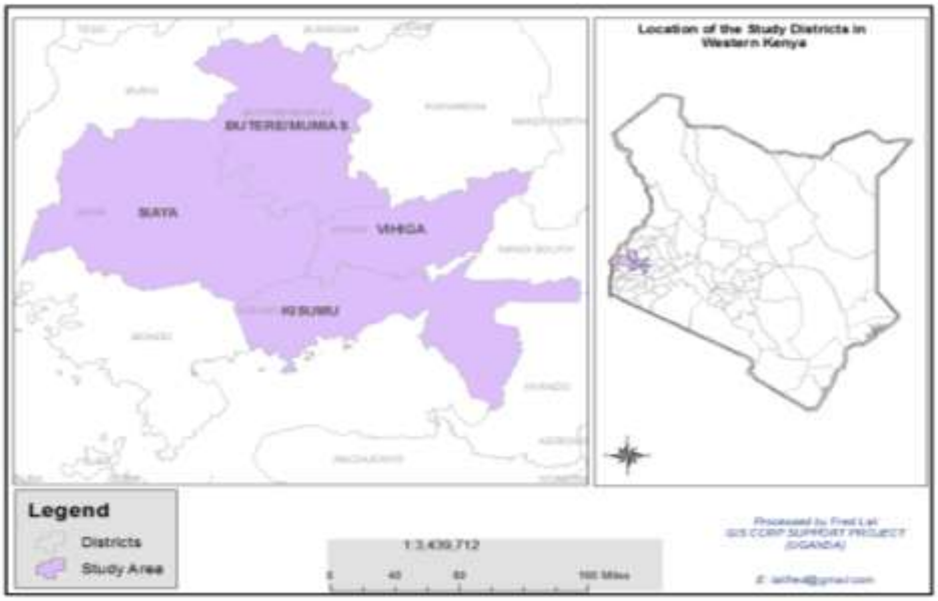

Figure 1. Map showing study sub-counties (districts) in western Kenya.
org/10.1016/j.jscs.2010.06.006.

(Midega *et al.,* 2015b), with only a small proportion planting medium maturity hybrids WH505.

Sampling and determination of incidence and severity of maize ear rot

A total random sample of 224 maize plots was picked from a sampling frame comprising push-pull and maize monocrop plots of equal number (112) in the study sub-counties. Each plot was surveyed for ear rot incidence and severity through a randomized sampling process during each cropping season, short rain and long rain. Siaya sub-county was however not surveyed during the second cropping season as only a few farmers planted maize during the season. At the beginning of harvest, 100 maize cobs were randomly picked per plot from which ear rot incidence was determined by physical count as described by Mutitu et al. (2003). A score of severity on a scale of 0-5 where: 0= No infection, 1=1-10%, 2=11-25%, 3=26-50%, 4=51-75% and 5=76-100% infection was used to estimate severity (Jeffs, 2002). Compendium with well-illustrated photographs of maize ear rots was used and tested before identification and characterization. Samples of infected (symptomatic) and clean (asymptomatic) maize ear were also collected and transported in a coolbox to the laboratory at the international centre of insect physiology and ecology (*icipe*), Thomas Odhiambo Campus at Mbita Point in western Kenya. These samples were further dried in an open air to moisture level of 13% measured using moisture meter (Model KM-36G, AWR Smith Process Instrumentation cc, South Africa). Samples were then hand shelled and milled before storage in a refrigerator (-4°C), waiting further mycotoxin analysis.

Mycotoxin extraction and analysis

Mycotoxin extraction and assay was conducted using ELISA commercial kit (Helica Biosystem Inc., Fullerton, CA, USA) for total aflatoxin, Cat. No.941AFLO1M-96; total fumonisin, Cat. No.951FUM01C-96; zearalenone, Cat. No.951ZEA01N-96; and deoxynivalenol, Cat. No.941DON01M-96 as described by Gutleb et al. (2015). A total of 20 mg of sub-samples of maize flour was extracted in 100 ml of solvent. Total aflatoxin was extracted in 70% methanol, total fumonisin and zearalenone in 90% methanol, and deoxynivalenol in distilled water. The mixture was blended and filtered with Whatman filter paper number one. Filtrate was used to measure for total aflatoxin directly, but diluted with distilled water for total fumonisins (1:20) and deoxynivalenol (1:10), and 70% methanol for zearalenone (1:10). The ELISA assay was conducted according to manufacturer's instructions. Optical density (OD) of the reaction for AFB, FB, ZEA and DON was measured using a microplate reader (*EZ* Read 400, biochrom) at a wavelength of 450 nm. Standard curve for each mycotoxin tested was generated using OD of five standards with known concentration as provided in the kit. Test (unknown samples) value was then determined by interpolation from the standard curve. Sample was tested within the range of 1-20 ppb for total AF; 100-6,000 ppb, total FB; 15-500 ppb, ZEA; and 500-10,000 ppb, DON. Sample which had exceeded upper limit of quantification was subjected to additional extract dilution. The final result was converted from parts per billion (ppb) to equivalent microgram per kilogram (µg/Kg).

Data analysis

Effects of cropping system and season on ear rot incidence were analyzed using generalized linear model, while ear rots severity were analyzed by analysis of variance using R software version 3.3.1 (R Core Team, 2013). Mean, frequency and percentage of samples contaminated with mycotoxins were presented by simple descriptive statistic using SPSS version 22 (IBM Corp, 2013).

RESULTS

Effect of cropping system on incidence and severity of maize ear rots

The study revealed that cropping system had a significant effect ($p \leq 0.001$) on the incidence of ear rots as shown in Table 1. Generally, there was high total ear rot incidence (20.8%) observed with sole maize than with push-pull system (7.3%). Similarly, there were significantly higher ($p \leq 0.001$) incidences of each of the ear rot types with sole maize than with push-pull. The sequence of incidence of ear rot types in sole maize and push-pull from highest to lowest was as follows: *Diplodia* < *Gibberella* < *Aspergillus* < *Fusarium* < *Penicillium*. The respective incidences of ear rots in sole maize and push-pull were 7.31 and 3.33%, *Diplodia*; 4.48 and 1.30%, *Gibberella*; 2.09 and 0.65%, *Aspergillus*; 0.51 and 0.21%, *Fusarium*; and 0.40 and 0.11%, *Penicillium*. The results in Table 2 showed that the severities were also significantly different ($p \leq 0.001$) between the two cropping systems; however, they seemed to be influenced by incidence of the ear rots. For instance, *Diplodia* and *Gibberella* ear rots had the highest severities of 1.85 and 1.15 in sole maize, and 0.84 and 0.62 in push-pull, when compared to other ear rots. However, for *Aspergillus* and *Fusarium* ear rot this seemed not to be the case, as *Aspergillus* had higher incidence than *Fusarium*, yet it had the lower severity rating (0.25 and 0.09) than *Fusarium* (0.68 and 0.19) with sole maize and with push-pull, respectively. Conversely, insignificant and least severities of 0.05 and 0.03 were reported in *Penicillium* ear rot with sole maize and push-pull, respectively.

Influence of cropping season on incidence and severity of maize ear rots

Short rainy season reported the highest (16.0%) incidence of total ear rot compared to the long rainy season (10.1%) (Table 1). However, incidence of *Penicillium*, *Diplodia* and aspergillus ear rots ear rots was significantly higher ($p \leq 0.01$) in the long rainy season than in the short rainy season. Conversely, incidence of *Diplodia* ear rots was significantly higher ($p \leq 0.001$) in the short rainy season than in the long rainy season. In terms on incidence levels, the highest ear rot incidence was recorded in *Diplodia* in both short (8.02 %) and long (3.12 %); while the lowest incidence was recorded in *Penicillium* in the short (0.05 %) and long (0.7 %) rainy seasons. Severities of ear rots were mostly insignificant with season, except for *Penicillium* ($p \leq 0.043$). There was no significant observable interaction between cropping system and season on incidence and severity of all types of ear rots (Tables 1 and 2).

Ear rots and mycotoxin incidence levels

Incidence of mycotoxins was higher in symptomatic than asymptomatic ears samples (Table 3). Based on mycotoxin type, the order of decreasing incidence on symptomatic samples was zearalenone (ZEA) (100%), total aflatoxin (AFB) (93.3%), deoxynivalenol (DON)(80.0%) and fumonisins (FB) (65.9%). However, asymptomatic ear samples had unexpected high incidence of ZEA (90.3%) and DON (51.6%). The mycotoxin ranges were also wider in symptomatic (ZEA, 18.7-688 µg/Kg, AFB, 0.35-28.9 µg/Kg; DON, 0-18,260 µg/Kg; and FB, 0-8,280 µg/Kg) than in asymptomatic (AFB, 0-11.7 µg/Kg; DON, 0-4,360 µg/Kg; FB, 0-6,460 µg/Kg; and ZEA, 0-405.8 µg/Kg) samples. The respective average levels of four quantified mycotoxins, ZEA, AFB, DON and FB, were 274.3, 6.1, 3,672 and 4,193.9 µg/Kg, respectively on symptomatic samples, showing high levels of mycotoxins compared to 48.4, 0.39, 633.9 and 1,616.8 µg/Kg on asymptomatic ears samples. The incidences of samples with mycotoxins beyond acceptable levels were also high; higher on symptomatic ears with 46.7, 28.9, 50.0 and 56.8% reported for ZEA, AFB, DON and FB, respectively. On asymptomatic samples, incidences were as low as 3.2% for AF and ZEA, and 19.4% for DON and FB.

DISCUSSION

Maize ear rots reduce grain yields and quality, with some of the causative pathogenic fungi producing mycotoxins that pose health risk to humans and livestock (Mukanga et al., 2010). Such ear rots are thus an important component of the myriad factors responsible for the high rates of food insecurity and health complications among smallholder farm families in sub-Saharan Africa. There is evidence that attack of maize by the ear rots and mycotoxins begin before the crop is harvested (Mukanga et al., 2011), and the attack is aggravated by grain handling and storage conditions (Mutiga et al., 2015). Indeed, incidence of ear rots in the study region, pre-harvest, often exceeds 20% (Ajanga and Hillocks, 2000), as confirmed by the current study. Notably, results of the current study, which to the best of our knowledge is the first study that directly relates ear rots and mycotoxins with cropping system under field conditions, demonstrated that maize grown under the push-pull cropping system suffered significantly less ear rots than sole maize, reducing the incidence level to 7.3%.

Attack of maize by stemborer pests has been shown to predispose the grains to ear rots and mycotoxin attack. Studies by Ajanga and Hillocks (2000) reported positive and high correlation between stemborers and incidence of ear rots in maize. Additionally, an interplay of other factors such as increase of organic matter (Alakonya et al., 2008), cover cropping (Tédihou et al., (2012), and intercropping (Vincelli, 1997; Flett and Ncube, 2015) have been reported to reduce ear rot incidence in maize. The push-pull cropping system effectively controls stemborers in maize (Khan et al., 2014; Midega et al., 2015a, b), improves soil organic matter content (Midega et al., 2005) and provides other soil improvement benefits. Therefore

Table 1. Effects of cropping system, season and their interaction on percentage incidence of ear rot disease.

Factor	Level	Gibberella x̄±SE	Fusarium x̄±SE	Penicillium x̄±SE	Aspergillus x̄±SE	Diplodia x̄±SE	Total incidence x̄±SE
System	Push-Pull (PPT)	1.30±0.1	0.12±0.1	0.11±0.04	0.65±0.1	3.22±0.2	7.30±0.3
	Maize Monocrop (MM)	4.48±0.2	0.51±0.2	0.40±0.1	2.09±0.2	7.31±0.3	20.8±0.4
Season	Long rains (LR)	2.02±0.1	2.67±0.1	0.70±0.1	1.38±0.1	3.12±0.2	10.1±0.3
	Short rains (SR)	3.00±0.2	0.22±0.2	0.05±0.04	0.96±0.1	8.02±0.3	16.0±0.4
System x Season							
	PP- LR	3.21±0.2	0.20±0.1	0.22±0.1	1.58±0.15	4.7±0.2	13.9±0.4
	PP- SR	2.19±0.2	0.26±0.1	0.21±0.03	1.05±0.14	4.93±0.3	12.1±0.3
	MM –LR	2.19±0.2	0.26±0.2	0.21±0.1	1.05±0.2	4.93±0.3	12.1±0.5
	MM-SR	2.19±0.3	0.26±0.3	0.21±0.1	1.05±0.2	3.93±0.5	12.1±0.6
Source of variation							
System		***	***	***	***	***	***
Seasons		Ns	***	***	**	***	***
System x Season		Ns	Ns	Ns	Ns	Ns	Ns

LR = Long rain; SR = Short rain; MM=Maize Monocrop; PP=Push-Pull; ns=not significant; x=Interaction,
x̄±SE, Standard error of the mean.Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05.

Table 2. Mean severity of ear rots disease by cropping system, season and their interaction.

Factor	Level	Gibberella x̄±SE	Fusarium x̄±SE	Penicillium x̄±SE	Aspergillus x̄±SE	Diplodia x̄±SE
System	Push-pull	0.62±0.09	0.19±0.05	0.03±0.01	0.09±0.03	0.84±0.1
	Maize momocrop	1.15±0.09	0.68±0.04	0.05±0.01	0.25±0.03	1.85±0.1
Season	Long rains	0.82±0.10	0.45±0.04	0.06±0.01	0.19±0.03	0.89±0.1
	Short rains	0.95±0.09	0.41±0.04	0.01±0.01	0.14±0.03	1.79±0.1
System x Season						
	PP – LR	0.58±0.1	0.23±0.06	0.06±0.02	0.11±0.04	0.45±0.1
	PP – SR	0.66±0.1	0.16±0.06	0.004±0.02	0.06±0.04	1.22±0.1
	MM-LR	1.06±0.1	0.68±0.06	0.07±0.02	0.27±0.04	1.32±0.1
	MM-SR	1.23±0.1	0.67±0.06	0.02±0.02	0.22±0.04	2.37±0.1
Source of variation						
System		***	***	Ns	***	***
Seasons		Ns	Ns	**	Ns	Ns
System x Season		Ns	Ns	Ns	Ns	Ns

LR = Long rain; SR = Short rain; MM=Maize Monocrop; PP=Push-Pull; x=Interaction; Ns=not significant;
x̄±SE, Standard error of the mean.Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05.

Table 3. Mycotoxin incidence and levels on symptomatic and asymptomatic ear samples.

Mycotoxin	Symptomatic ears				Asymptomatic ears			
	AFB	DON	FB	ZEA	AFB	DON	FB	ZEA
Total sample (N)	45	30	44	45	31	31	31	31
Positive N (%)	29 (93.3)	24 (80.0)	29 (65.9)	45 (100)	1 (3.2)	16 (51.6)	12 (38.7)	28 (90.3)
Range (µg/Kg)	0.35-28.9	0-18,260	0-8,280	18.7-688	0-11.7	0-4,360	0-6,460	0-405.8
Average (µg/Kg)	6.1	3,672	4,193.9	274.3	0.39	633.9	1,616.8	48.4

N, Number of ears sample; (%), Percent; AFB, Total Aflatoxin; DON, Deoxynivalenol; FB, Fumonisins (Total fumonisins B1+B2+B3); and ZEA, (Zearalenone); ML, Maximum Limit of concentration for mycotoxins.

the significant reduction in incidence of ear rots observed in the push-pull plots might have resulted from the multiple ecological benefits provided by the technology.

Planting seasons are important on disease forecasting and appropriate for decision by farmers (De Wolf et al., 2003). Maize is grown in seasons which have varied amount of rainfall and temperature, the two major factors for ear rot incidence and severity. In Uganda, Diploda was the most abundant ear rot found in areas receiving high rainfall (Bigirwa et al., 2006), thus Bigirwa et al., (2007) reported more ear rot during first season. There was similar observation in study, but during the second season and not first season which received high rainfall. This may be due to wet conditions at silking stage favourable for *Diplodia* and *Gibberella* infection and progression (Miller, 2001; Woloshuk and Wise, 2010); which was met when late rainfall cessation extended beyond silking stage in short rain (Mugo et al., 2016). Similarly, push-pull cropping system could as well promote cooler conditions due to high evapotranspiration from intercrop; thereby predisposing ears to potential infection with *Diplodia* or *Gibberella* ear rot as observed on insignificant by slightly high *Gibberella* and total ear rot incidence by interaction of push-pull and long rain season.

Fusarium mycotoxins are abundant in cereals and their products (Yazar and Omurtag, 2008), and are diverse in nature. They can cause food poisoning upon ingestion. Deoxynivalenol poisoning is characterized by diarrhea, vomiting, nausea, headache, dizziness and fever (Zain, 2011), while zearalenone is known to cause reproductive problems mostly in pigs, sheep and human beings (de Rodriguez et al., 1985; Smith et al., 1986; Kuiper-Goodman et al., 1987).

These two mycotoxins have received little attention due to their causal agents' devastation on wheat (Zain, 2011) than in maize, and less acute outbreaks compared to aflatoxin (Darwish et al., 2014) and fumonisin (Fadhohan et al., 2003) in the Sub-Saharan region. However, a likelihood of high population exposure might be seen from high average levels of zearalenone (3,663 µg/Kg) and deoxynivalenol (23,586 µg/Kg) tested from household maize samples in Tanzania (Degraeve *et al.,* 2016). Similarly, these two mycotoxins were found in high amount from our samples, with difference of less amount of mycotoxin on asymptomatic ear compared to symptomatic.

Conclusion

Conclusively, impact of cropping system on ear rot was evident and the system should be integrated with other management system to control ear rots and mycotoxins. The high incidence and amount of zearalenone and deoxynivalenol in these studies and elsewhere in sub-Saharan suggests that there is need for their surveillance by screening their presence and sensitizing of farmers on their management

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Control of broadleave and grass weeds in *Saccharum officinarum* with the use of pre-emergence herbicides

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In order to identify combinations of effective preemergent herbicides for weeds in sugarcane, an experiment was conducted at the Agricultural School of Concepción; Concepción, Paraguay. The experimental model was subdivided plots, with two factors: A_1 without herbicide for broadleaf weeds, A_2 diuron (dose 3 t ha^{-1}), A_3 atrazine (dose 4 kg ha^{-1}), A_4 sulfentrazone (dose 1.2 t ha^{-1}) and B_1 without herbicide for weeds, B_2 trifluralin (dose 3 l ha^{-1}), B_3 acetochlor (dose 1 l ha^{-1}), forming twelve treatments with three repetitions. They were applied in preemergence, where the evaluations were from 20 to 41 days after application (DDA), for: degree of control, residual effect and control of predominant weeds. The combination of diuron and acetochlor, produced high weed control (more 90% up to 41 days DDA), being superior to the other treatments. *Sida* spp was better controlled with diuron; whereas *Mollugo verticillata* was effectively controlled with most combinations; *Ipomoea nil* was more sensitive to sulfentrazone, while; *Cenchrus echinatus* was effectively controlled, with the combination of diuron plus acetochlor. It is concluded that with the combination of diuron and acetochlor, a large variety of weeds was controlled and the highest residual effect was recorded. In addition, atrazine combined with graminicides (trifluralin or acetochlor) or diuron with acetochlor, provided total control of weeds. It is recommended: for *Sida* spp. diuron with acetochlor or atrazine. For *Mollugo verticillata*, sulfentrazone alone or combined with graminicides. *Ipomoea nil*, sulfentrazone. *Cenchrus echinatus*, diuron and acetochlor combined.

Key words: *Saccharum officinarum*, weeds, preemergence herbicides.

INTRODUCTION

In Paraguay, according to CAN (2008), 95.3% of the farms have at least a small area dedicated to the cultivation of sugarcane; In the department of Concepción, this item is essential in rural farms. The initial development of the sugarcane plant is slow; therefore, if the weeds are not removed during this critical time; determined according to López (1988), in the first 35 to 90 days after implantation the population and crop

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production can be reduced by up to 40% (Toledo and Cruz, 2012).

Weeds compete with crops for water, light, and nutrients. Sugarcane is especially sensitive to competition with invasive plants, and according to Valle et al. (2000), if an efficient and timely control is not carried out, production may be affected, as well as behaving as host of pests.

The use of herbicides is replacing the mechanical and manual methods of weed control, since it offers advantages such as: its rapid application, safety for cultivation, effectiveness for control, solution to the lack of labor and prolonged residual power.

It is in this context that we propose to establish some strategies to give a solution to the problems of weeds, because consequently they can cause the reduction of sugarcane production. This measure is to present a technology, that is, an effective alternative for the control of invasive plants. This would involve the application of combined herbicides with other chemical bases, for the eradication of invasive plants.

In this research work, the objective was to identify the combinations of preemergence herbicides that are most effective for the control of *Sida* spp, considered by Díaz (2003), of the main weeds in sugarcane; *Mollugo verticillata*, Arias and Salazar (1999), mention it among the most abundant weeds in the cultivation of sugarcane. *Ipomoea nill*, counted among the worst weeds of sugarcane (Roncaglia and de Marco, 1995; Cordova et al., 2014); *Cenchrus echinatus*, cited among the most important weeds in sugarcane crops (Valle et al., 2000) weeds and broadleaf weeds in the cultivation of sugarcane.

MATERIALS AND METHODS

The experiment was conducted at the Agricultural School of Concepción, (23° 22' 44.77"S and 57° 23'26.64" W). The average temperatures vary between 23 and 17°C, with maximum of 41°C in the summer season and minimums of 5°C in the winter season, with slight incidences of frost. The annual average precipitation is around 1500 mm (DINAC, 2016). The soil of the region is characterized as clear thin sandy texture with weak structure. López et al. (1995). The experimental design used was subdivided plots (4 × 3), consisting by 12 treatments with 3 replicates, which were laid out in 4 main plots and divided into 3 sub plots for the application of treatments. Each experimental unit consisted of 3 rows (or rows) of 6 m in length and 1.20 m between each of them. This represented a surface area for the experimental units of 21.6 m^2. The total experimental area was 950 m^2. The treatments were, Control ($A_1 + B_1$), trifluralin ($A_1 + B_2$), acetochlor ($A_1 + B_3$), diuron ($A_2 + B_1$), diuron + trifluralin ($A_2 + B_2$), diuron + acetochlor ($A_2 + B_3$), atrazine ($A_3 + B_1$), atrazine + trifluralin ($A_3 + B_2$), atrazine + acetochlor ($A_3 + B_3$), sulfentrazone ($A_4 + B_1$), sulfentrazone + trifluralin ($A_4 + B_2$), sulfentrazone + acetochlor ($A_4 + B_3$). The choice of this experimental area was taking into account the infestation of weeds. The preparation of the site was carried out in a conventional way, by using a heavy harrow, followed by cleaning and levelling of the land to leave a smooth seedbed. For sowing, the canes were cut, leaving stakes with 3-4 buds, which were later deposited and distributed in the furrows to approximately 0.3 m depth. The herbicides were applied out immediately after planting and following precipitation, preemergence to the weeds using a backpack sprayer, of 20 L of capacity, which works at an approximate pressure of 5 Kg/cm²; being necessary approximately 10 charges for the coverage of 1 ha. The herbicides were applied individually, in order to avoid later problems such as those cited by Urrretabiscaya et al. (2016), which mention problems such as: incompatibility of mixtures with phase separation, clot formation, coalescence, flocculation, sedimentation; because of: low volume of water (higher concentration), hard water (affect the emulsifier), low temperature (lower solvent capacity of the water and slow chemical reaction speed), rapid spillage of the agrochemical (little time to dissolve), poor agitation (little return or misplaced), and finally an incorrect mixing order. The concentration of active ingredient and dose for each of the products applied was shown in Table 1. The evaluated parameters were:

Table 1. Concentration of active ingredient and dose of herbicides used.

Products	Concentration (%)	Dose	Unity
Trifluralin	50	3	$L.Ha^{-1}$
Acetochlor	84	1	$L.Ha^{-1}$
Diuron	50	3	$L.Ha^{-1}$
Atrazine	75	4	$Kg.Ha^{-1}$
Sulfentrazone	50	1.2	$L.Ha^{-1}$

Weed control: The evaluations were carried out at four intervals; at 20, 27, 34 and 41 days after treatment by quantifying emerged weeds compared to the control by means of a 0.25 m^2 quadrat. The percentage of controlled weeds was recorded according to the scale of Truelave (1977).

Residual effect: The period during which the herbicides had pre-emergence control of the weeds was evaluated by quantifying weeds that emerged in the matched control, as in the plots that received the applications. The evaluations were made at 20, 27 34 and 41 DDA.

Control of the predominant weeds: The most dominant weeds were evaluated by quantifying them compared to the control. Data were subjected to analysis of variance (ANOVA) using the Test F (5%) and significant effects were subjected to a comparison of means by Tukey (5%).

RESULTS AND DISCUSSION

Weed control

In the absence of herbicides for grassy weeds (B_1), the herbicides diuron and atrazine resulted in weed controls of 80 and 79% respectively, being superior to the control obtained with sulfentrazone (65%). Faced with a high population of broadleaf weeds in the experimental plot, the high degree of control obtained with selective broadleaf herbicides can be attributed to the efficacy of the product and its high residual power. Also, Esqueda, (1998) obtained 87% control of broadleaf weeds with the

Table 2. Comparison of averages for the determination of weed control (%) with the use of preemergence herbicides in *Saccharum officinarum*.

Factor A	Weed control (%)		
	Factor B		
	Without Herbicide	Trifluralin	Acetochlor
Without Herbicide	$0^{cC(1)}$	24^{cB}	37^{cA}
Diuron	$80^{a B}$	82^{abB}	92^{aA}
Atrazina	79^{aB}	89^{aA}	71^{bC}
Sulfentrazone	65^{bB}	79^{bA}	72^{bAB}

([1]) Equal letters, lowercase in columns and uppercase in rows, indicate statistical equality through the Tukey test with 5% error probability. Factor A: herbicide for the control of broadleaf weeds; Factor B: herbicide for the control of fine leaf weeds.

application of atrazine in sugarcane.

In combination with the graminicide trifluralin (B_2), atrazine resulted in 89% weed control, being significantly superior to the control obtained with the herbicide sulfentrazone. The diuron showed a good control of 82%, but statistically similar to sulfentrazone with 79%. Combined with acetochlor (B_3), the herbicide diuron resulted in the highest percentage of weed control (92%), being significantly higher than those obtained with the other herbicides (atrazine and sulfentrazone). Alfaro et al. (2002), obtained adequate levels of control (more than 90%), with the combination of hexazinone, diuron and 2,4-D + picloran; results similar to those achieved in this investigation with the combination of diuron and acetochlor.

The use of selective herbicides for broadleaves, either alone or combined with graminicide herbicides, resulted in a high percentage of weed control, with all cases being significantly superior to the control without herbicide (Table 2). When the diuron treatment was used, the herbicide acetochlor was found to have the highest percentage of control (92%), being significantly higher than that obtained from the combination of diuron with the herbicide trifluralin, which did not present any additional control. According to Guevara (2014), some pre-emergent herbicides such as diuron, are used in combination with Acetochlor, to extend the spectrum of control.

In combination with atrazine (A_3), the herbicide trifluralin resulted in the highest percentage of weed control of 89%, being significantly higher than that obtained with acetochlor. The degree of weed control with atrazine decreased significantly with the application of acetochlor (71%); being these data superior to those reached by Alfaro et al. (2002), who obtained 54% control, with the mixture of atrazine, ametryn and 2,4-D + picloran).

In combination with sulfentrazone (A_4), trifluralin showed the highest percentage of weed control (79%), being significantly higher than the control percentage of acetochlor. Cantamutto et al. (1996), report that in works on early application of melon cultivation herbicides, trifluralin, made control of weeds, both broadleaved and grasses. With respect to the use of selective herbicides for fine leaf weeds, individually they do not demonstrate high percentages of control. In combination with herbicides for broadleaf weeds, in general, they seem to enhance the effects of these, with the exception of acetochlor with atrazine, which decreases the control exerted individually by atrazine. This may be due to the lower incidence of grasses in the total weed population, and in some cases due to the decrease in the phytotoxic effect of graminicides as a consequence of mixtures with some of the broadleaved herbicides.

Residual effects

Table 3 shows the residual effect (%) in the different treatments. In general, the graminicides showed low levels of weed control until 20 days after their application, reaching the highest values of control with acetochlor (66%). The percentage of weed control of the graminicide herbicides decreased to levels below 50% from 27 days after the application (DAA). These results indicate that the graminicides had a rather deficient effect until 20 days after its application. However, Villegas, (2011) in an experiment carried out to control weeds in chia cultivation, showed that trifluralin offers good control until 20 days after application. On the other hand, Alfaro et al. (2002), I observe a reduction of the residual power of acetochlor decreasing of 81.43% at 75 days after the application; up to 10.16% at 90 days after the application. With the use of diuron as a herbicide for broad leaves, without combination with other herbicides a good residual effect was obtained up to 34 DDA, reaching very good levels of weed control, higher than 80%; from which it progressively decreased its control effect until reaching 59%. González et al. (2018), with the use of diuron in sugary sorghum culture, I observed a 70% weed control, at 35 days after the application; maintaining these levels of control until 50 days after the application.The herbicide diuron substantially improved the percentage and the residual period of weed control when it was combined

Table 3. Comparison of averages for the determination Residual effect (%) of the herbicides used to control weed in *Saccharum officinarum* at 20, 27 34 and 41 days after application (DAA).

Treaments	Days after the application (DAA)			
	20	27	34	41
Control	0	0	0	0
Trifluralin	42 to C	33 aD	10 bFG	10 bD
Acetochlor	66 to B	46 bD	26 cF	9 dD
Diuron	85 aA	90 aAB	85 aABC	59 bC
Diuron + Trifluralin	93 aA	93 to AB	78 b ABCD	65 bC
Diuron +Acetochlor	87 aA	97 a A	92 aA	94 to A
Atrazine	91 aA	81 abBC	74 bBCD	70 bBC
Atrazine +Trifluralin	87 aA	94 aAB	90 aAB	83 aAB
Atrazine +Acetochlor	67 aA	70 BC	73 to CD	75 aBC
Sulfentrazone	61 bcB	83 aABC	50 cE	66 bC
Sulfentrazone + Trifluralin	91 aA	93 aAB	64 bOF	67 bBC
Sulfetrazone +Acetochlor	59 bB	80 BC	76 a ABCD	73 Bc

Equal letters, lowercase in columns and uppercase in rows, indicate statistical equality through the Tukey test with 5% error probability.
Factor A: Herbicide for the control of broadleaf and fineleaf weeds; Factor B: Moments of evaluation.

with the graminicide herbicide acetochlor, reaching 94% control of the at 41 days after application. Diuron combined with trifluralin had a high weed control until 27 days after its application (93%), after which it gradually decreased its residual effect until reaching moderate levels of weed control at 41 DAA (65%). Similar results of the residual effect of the herbicide diuron was found by Esqueda (2005) who verified a level of weed control higher than 95% up to 30 DAA, which decreased slightly with time until 80% of weed control was reached. 90 days after its application. Metzler and Ahumada (2013) also observed that there was a moderate level of 80% weed control with the use of diuron.

With the use of atrazine without combination with graminicides, the highest percentages of weed control were obtained up to 27 DAA (higher than 80%), significantly decreasing its effect from 34 DAA until reaching moderate levels of weed control (around 70%). Gonzalez et al. (s.f.), could observe that the residual effect of atrazine remained at control levels of 50%, between 35 and 100 DAA. Atrazine significantly increased its residual effect when combined with trifluralin, reaching high weed control percentages (above 80%) until 41 days after its application. However, when atrazine is combined with acetochlor, its degree of weed control significantly decreased to moderate levels (around 70%) but constant until the end of its evaluation (41 DAA). According to Metzler and Ahumada (2013), in an investigation on the control of weeds with preemergence herbicides, using atrazine and making observations at 30 DAA up to 70 DAA, obtained control of 85% of weeds.

With the use of sulfentrazone moderate control of the weeds was obtained (around 80%) up to 27 DAA; in this sense Diez (2013), mentions that the residual effect of sulfentrazone, is variable in some cases has no residual effects on the soil to be absorbed into organic matter and soil clays, in addition to being sensitive to microbial decomposition, while in other cases it can remain active for 3 to 8 weeks. Control by sulfentrazone was significantly increased to levels above 90% when in combination with trifluralin but did not increase its residual effect at 27 DAA. Sulfentrazone combined with acetochlor showed a moderate degree of weed control (greater than 70%) during the entire evaluation period of the experiment (41 DAA).

Control of predominant weeds

Table 4 shows the control of predominant weeds (%) in the different treatments.

Control of *Sida* spp

All the herbicides for grass weeds applied resulted a very low percentage of mallow control. However, in mixtures of diuron with acetochlor and the use of atrazine alone or in a mixture with acetochlor resulted in the highest percentages of control of the mallow, reaching levels of 100% and close to 100%, respectively. The diuron only showed moderate control of the mallow (75%). However, when this herbicide was mixed with graminicides, the control percentage of the mallow increased to very good levels, reaching 88% when it was mixed with trifluralin

Table 4. Comparison of averages for the determination of control for predominant weeds (%) with preemergence herbicides in *Saccharum officinarum.*

Treaments	*Sida* spp	*Mollugo verticillata*	*Ipomea nil*	*Cenchrus echinatus*
Witness	0	0	0	0
Trifluralin	28aD	88aB	43aD	83aB
Acetochlor	13bD	48bD	28bD	43bD
Diuron	75cC	97bB	25cD	84cB
Diuron+Trifluralin	88bB	92cB	75aC	88bB
Diuron+Acetochlor	100aA	100aA	50bC	94aB
Atrazine	99aA	88bB	69bC	56aC
Atrazine+Trifluralin	75bC	100aA	74aC	40bD
Atrazine+Acetochlor	98aA	100aA	69bC	40bD
Sulfentrazone	78aC	100aA	92aB	40bD
Sulfentrazone+Trifluralin	29cD	100aA	38cD	65aC
Sulfentrazone+Acetochlor	70bC	100aA	76bC	30cD

Equal letters, lowercase in columns and uppercase in rows, indicate statistical equality through the Tukey test with 5% error probability. A: Full effect (Excellent weed control); B: Severe effect (Very good weed control); C: Moderate effect (Moderate weed control); D: Light effect (Poor weed control); E: No effect (Very poor weed control).

and 100% of control when combined with acetochlor. This of diuron with acetochlor resulted in control percentage significantly higher than those obtained with diuron alone or mixed with trifluralin. Lorenzi (2014) found that *Sida* spp is highly susceptible to diuron with more than 95% control; although these results do not correspond to what was obtained in the current investigation since the herbicide had inferior results.

The use of atrazine alone or in a mixture with acetochlor showed high percentages of control of *Sida* spp (99 and 98%, respectively). However, the control by atrazine was negatively affected when this herbicide was mixed with trifluralin as control decreased to 75%. Atrazine, according to Lorenzi (2014), shows good control of more than 90%, which confirms the results of this research work. Sulfentrazone only showed a moderate degree of control of *Sida* spp (78%), but this lost its effect when it was mixed with both graminicides, namely acetochlor and trifluralin.

Control of *Mollugo verticillata*

Trifluralin, showed good control of *M. verticillata* (82%), being significantly greater than the control by acetochlor. Sulfentrazone, individually or combined with Trifluralin and Acetochlor; the Diuron combined with Acetochlor; and Atrazine combined with Trifluralin and Acetochlor, showed 100% control of this weed. Diuron, either alone or in a mixture with the graminicides, showed control of *M. verticillata* greater than 90%. Diuron in a mixtures with acetochlor obtained the highest percentage of control of *M. verticillata* (100%), being significantly greater than that obtained with diuron alone (97%) or the mixture of diuron with trifluralin (92%). Esqueda (1999), investigating with different mixtures of herbicides in pre and post emergency, in the sugarcane crop, could observe total control of *M. verticillata*, up to 90 DDA, with the combined use of diuron + hexazinone and ametryn + atrazine, applied in preemergence. Atrazine had a high control percentage of 88% of *M. verticillata*, whose effect was significantly increased with the combination with the herbicides trifluralin and acetochlor, reaching 100% control.

Control of *Ipomoea nil*

Sulfentrazone resulted the highest percentage of control of *Ipomoea nil* (92%), being significantly higher than those obtained by the single applications of diuron and atrazine, whose control percentages was 25 and 69% respectively. These results are in agreement with the work carried out by Fernández et al. (2011) for the control of the weed *Ipomoea purpurea* with preemergence use of sulfentrazone, where controls of 90% was obtained in a soybean crop. Likewise, Canaza and Fernandez (2016), using sulfentrazone in combination with other herbicides in the soybean crop, observed 94.34% control for this weed at 10 DAA, decreasing this figure to 44% at 20 DAA; and being ineffective after 38 DAA.

The use of diuron and atrazine, combined with trifluralin, resulted in good percentages of control of *Ipomoea nil*, reaching 75 and 74%, respectively. This was significantly greater than results from these herbicides combination with acetochlor. The combination between sulfentrazone and acetochlor, resulted in control of *Ipomoea nil* at 76%, which was significantly greater than the combination with trifluralin.

Control of *Cenchrus echinatus*

Trifluralin resulted in the highest percentage of control of *C. echinatus*, at 83% being superior to the control obtained by acetochlor (43%) *C. echinatus* is highly susceptible at 100% control to the action of the herbicide trifluralin when applied preemergence (Lorenzi, 2014). With the use of diuron, either alone or in mixtures, the highest percentage of control of poaceae was obtained, being significantly higher than that obtained by atrazine and sulfentrazone at 56 and 40%, respectively. When the diuron was combined with acetochlor, the highest percentage of control of *C. echinatus* was obtained (94%), being significantly superior to the control obtained by diuron plus trifluralin at 88%. Atrazine alone or in combination with other graminicides herbicides showed a very low percentage of *C. echinatus* control of 56%. In combination with the graminicides, the control by atrazine of *C. echinatus* decreased, most probably due to problems of incompatibility of the herbicides mistures. Sulfentrazone application resulted in very low control of *C. echinatus* (40%). However, there was a significant increase in control when this herbicide was combined with trifluralin, reaching 65%. However, when the sulfentrazone is combined with acetochlor the percentage of control was reduced considerably to 30%, which indicates the occurrence of an antagonism between the herbicides.

Conclusions

With the use of preemergence herbicides, diuron with acetochlor, effectiveness was achieved for the control and reduction of a great variety of weeds. In addition, the same combination offers the best residual effect with 94% control until 41 days after application.

RECOMMENDATIONS

To obtain control of *Sida* spp*.* diuron with acetochlor or atrazine may be used. For the control of *M. verticillata*, sulfentrazone alone or combination with grass herbicides such as trifluralin or acetochlor are effective. In addition atrazine and its mixture with graminicides (trifluralin or acetoclor) or diurón with acetoclor, offers total control of the weed. In order to obtain control of *Ipomoea nil*, the use of sulfentrazone may achieve very good efficacy and thus reduce the impact that the weed might cause. Control of *C. echinatus*, will be obtained with the combination of diuron and acetochlor with which satisfactory effects will be achieved.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Distribution of rainwater by species of caatinga vegetation

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This study aimed to estimate the redistribution of rainfall in a Caatinga vegetation fragment of about 50 years in fallow system, in late stage of regeneration. To quantify the throughfall, rainfall collectors were installed in six individuals of the species *Croton blanchetianus*, *Mimosa tenuiflora*, *Cnidoscolus quercifolius*, *Aspidosperma pyrifolium* and *Poincianella bracteosa*, totaling 30 rainfall collectors. For the stemflow, collecting system set around the stems of the six subjects in each of the five selected species was used, consisting of collecting gutters in a spiral. In addition, a rain gauge was installed in an open location to quantify the open precipitation, which during the study period was equal to 1173.56 mm. The *C. quercifolius* species had the highest average of throughfall; probably this may be related to the peculiarities in relation to architecture and canopy shape. The stemflow has lower shares in gross precipitation in *A. pyrifolium* and *C. quercifolius* species, representing 15.25 to 12.48% of the total rainfall, respectively. The values obtained for the interception losses were greater than 60% in all species.

Key words: Forestal hydrology, throughfall, stemflow.

INTRODUCTION

The vegetation play a key role in the Earth System as it can control the soil erosion, the runoff generation, the infiltration process, and the soil properties (Cerdà and Doerr, 2005; Keesstra et al., 2009; Keesstra, 2007; Barua and Haque, 2013; Novara et al., 2011; Cadaret et al., 2016; Archer et al., 2016).

Rainfall participates in nutrient cycling, after contacting the forest canopy, as rainwater has its physical and chemical attributes modified by leaching of the metabolites of leaf tissues, stems and branches. In addition, rainfall washes the particles derived from dry deposition that subsequently accumulates in the dry

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period, being the broad-leaved species, the ones that have greater leaching, compared to the conifers (Oki, 2002).

Rain comprises, therefore, a significant compartment in the internal dynamics of nutrients in a forest ecosystem (Parker, 1983), delivering the nutrients that are dissolved to be absorbed by the roots.

During the water cycle, part of the volume of rainfall entering the ecosystem is retained by the leaves of the canopy, evaporating because of the temperature, solar radiation and wind. Another part reaches the forest floor, after washing the canopies and stems, with the possibility to suffer percolation or to be superficially drained (Delitti, 1995).

The percolated water, when exceeds the holding capacity of the soil, moves to the groundwater leaching some nutrients, leaving the ecosystem. Yet the water stored in the soil constitutes the soil solution where nutrients are in forms that are assimilable by plants, representing the reservation that plants will absorb to perform the metabolic activities (Britez, 1994).

The volume of water that cannot infiltrate the soil drains superficially. In high rainfall, when the volume and energy of the drained water are higher, fine soil particles are carried along with the water, leading to erosion, often intense, especially in areas with low vegetation, like the degraded areas of Caatinga. The plant biomass contributes to rainwater interception, providing its storage in the soil and the increase in infiltration rates (Olson, 1963).

Forest cover has significant influence on the redistribution of rainfall in all ecosystems, thus, the study of redistribution of rainwater in forest ecosystems is important for understanding the processes of interception, infiltration, percolation, uptake, transpiration and cycling of nutrients in forest ecosystems.

For Ferreira et al. (2005), the interception of rain by the plant canopy is a vitally important component to understand the hydrological cycle since this varies according to the morphological aspect of the vegetation (age, canopy, architecture), to the dominant precipitation regime in the region and the time of year. Yet the precipitation that runs through the canopy (incident rainfall) and reaches the forest floor depending on the nature and density of the vegetation, since this coverage temporarily retains certain amount of incident precipitation.

According to Bruijnzeel (1990), tropical forests, in general, reach variations of throughfall between 75 and 96%, the stemflow varies between 1 and 2%, and the interception by vegetation between 4.5 and 24% of the gross precipitation. However, it appears that the interception, internal precepitation and stemflow are parameters that have widely varying values within one or more ecosystems (Arcova et al., 2003; Ferreira et al., 2005; Oliveira Júnior and Dias, 2005; Germer et al., 2006; Oliveira et al., 2008; Medeiros et al., 2009; Izidio et al., 2013; Sousa et al., 2016).

The type of vegetation involves different characteristics in the soil and water properties in a given ecosystem; forest degradation has significantly favored the erosion in vast areas, with damage to hydrological processes and biodiversity (Freitas et al., 2013). Despite the relevance of the water study in forest areas, especially in degraded and susceptible to erosion' environments, there are few studies related to the distribution of rainwater in areas of Caatinga. This study aimed to estimate the redistribution of rainfall in a Caatinga vegetation fragment of about 50 years in fallow system, in late stage of regeneration.

MATERIALS AND METHODS

Study area

The research was conducted in an area of Caatinga located in Cachoeira of São Porfirio Farm's, Várzea district, microrregion of Western Seridó, Paraíba backwoods, placed in the coordinates 06° 48' 35" S and 36° 57' 15" W, with an average altitude of 271 m.

The climate of the study area is characterized as semi-arid, BSh' type (hot and dry) according to the Köppen classification. The average annual rainfall is between 400 and 600 mm, with a dry period of nine to ten months and average temperatures higher than 18°C in all months of the year; peak means around 33°C and minimum of 22°C (IBGE, 2002).

The study area soils are of crystalline origin, being shallow, stony and with high susceptibility to erosion, prevailing the association of Litholic Neosols, Luvisols and rocky outcrops.

The location where the research was implemented consists of an open arboreal-shrubby Caatinga vegetation with varying degrees of anthropism, with medium-low trees not exceeding 7.0 m in height. The natural vegetation of the area was cleared for the implementation of agricultural crops, mainly for cotton planting, being subsequently used as areas of goats and cattle grazing, occurring regeneration of the vegetation.

In the study area, we demarcated a transect 20.0 m × 50.0 m in size. The area has about 50 years of fallow, vegetation type in the most advanced stage of successional development, constituting a complex and with high floristic diversity community.

METHODOLOGY

In an open area located in about 200 m from the study area, the gross precipitation (P) was recorded by one (01) rainfall collector, made of wooden and polyethylene terephthalate (PET) gauges. The hand-made rain gauges are made up of a funnel with a circular opening of 9.2 cm in diameter and 2 L containers and installed 1.5 m above the ground (Figure 1a). The water accumulated in these rainfall collectors was conducted by hoses located at the lower end, this being stored in PET plastic containers fixed on the ground. This water is considered as a control, corresponded to the water that precipitated directly on the ground, without passing through the leaves or flowing through the stem.

Rainfall collectors like the previous ones were used to estimate the throughfall, distributed below the canopy of five Catinga species (*Croton blanchetianus* Baill, *Mimosa tenuiflora* Poiret, *Cnidosculus quercifolius* Pohl, *Aspidosperma pyrifolium* Mart, and *Poincianella bracteosa* (Tul.) L.P. Queiroz). These species were selected following the importance value (IV) determined by Sousa (2011). For each species, six replicates, totaling 30 individuals.

In each rain event, the stored precipitation volumes measured in

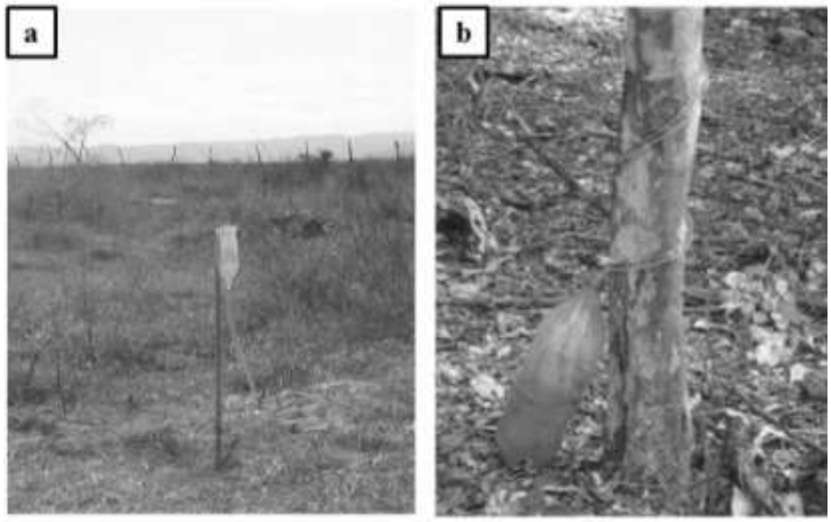

Figure 1. (a) Rainfall collector, (b) Stemflow collector.

milliliters in the field and, based on the dimensions of the containers, then converted into millimeters using the equation described in Gênova et al. (2007):

$$Precipitation = measured\ volume\ (mL) x\ 0.1504$$

For quantification of the stemflow, a collecting system set around the stems of the six individuals of each of the five selected species was developed, consisting of plastic hose of an inch, cut longitudinally, and thus becoming "collecting gutters" for the captation of the amount drained (Figure 1b). The collecting gutters were fixed in the form of spiral, following the circumference of the tree trunks, being used, for that, nails and glue based on cassava mass. Rainwater captured was directed to PET plastic containers positioned vertically and fixed to the ground, methodology adapted from Moura et al. (2009).

The stemflow was estimated in millimeters of water, by the mean of the volume stored in tanks and a conversion factor of 113.64 m², namely, due to its populational density, which has been estimated by dividing the values of size of the experimental area by the five species selected.

After obtaining the data, interception losses were estimated according to the equation defined by Helvey and Patric (1965):

$$I=P - (T + Sf)$$

where I = interception losses, P = gross precipitation, T = throughfall, and Sf = stemflow.

To study the behavior of throughfall, stemflow and standards of interception losses among species, were estimated for leaf area, canopy projection area (CPA), diameter at chest level (DCL) and basal area (g).

The determination of the leaf area was carried out considering the dimensions of 102 leaves of varying sizes including from the smallest to the largest sizes, which were collected randomly throughout the canopy of individuals of the species. The leaves collected were accommodated in cooler in order to avoid the loss of turgor during the determinations. The leaves were scanned on white background material, and for the direct measurement of leaf area, the digital images were manipulated using the ImageJ® Software (Powerful Image Analysis), calculating the actual total area of each leaf (Jadoski et al., 2012).

DCL measurement of each individual of the species studied was held using tape measure, used in determining the basal area of each individual. To study the canopy, four each individual canopies radii were measured, following the north-south-east-west orientation, getting the average canopy diameter (CD) for calculating the canopy projection area (Wink et al., 2012).

Statistical analyses

For the analysis of throughfall and stemflow among the studied species, first, the normality of the distribution was verified by the Lillifors test and the analysis of variance (ANOVA one-way) performed. Means were compared by 5% Tukey test. As for the analysis of throughfall and stemflow during the months studied, the species were analyzed separately.

RESULTS AND DISCUSSION

The gross precipitation data recorded in the study area shows the irregular distribution of rainfall during the study period (Figure 2).

The gross precipitation in the study area in the twenty-three months of study was estimated to be 1,173.56 mm. In 2011, the gross precipitation in the study area was 961.5 mm, as expected in the region, considering the average annual rainfall (300 to 1000 mm). The highest monthly rainfall was of 345.92 mm, corresponding to May/2011. In March/2012, only 4.00 mm was recorded.

No rainfall was recorded during the months of November/2010, July, September, November and December/2011. In addition, there was no water storage in rainfall collectors in the months of June and August/2011 and March/2012, which may be related to low rainfall that occurred in those months. Therefore, thirteen months of rainfall events was used in this study.

This distribution confirms the irregular pattern of rainfall

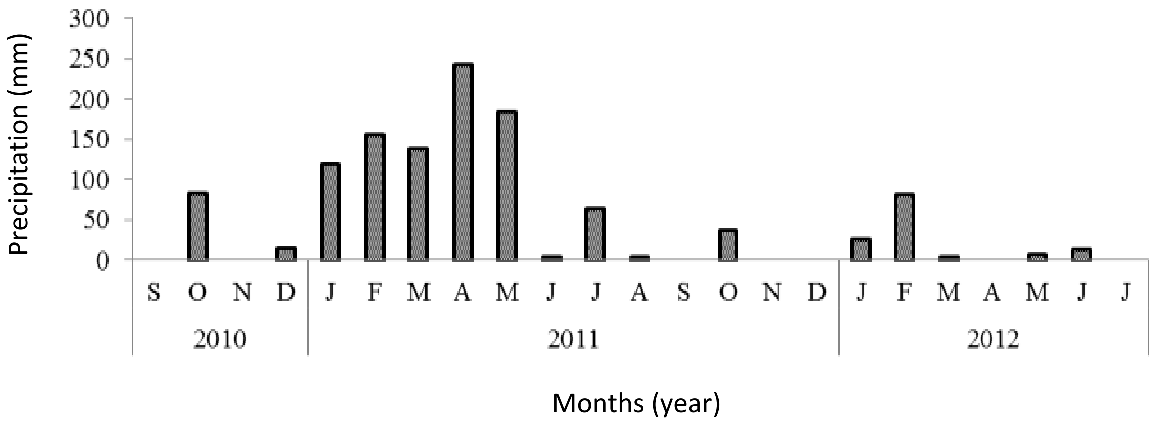

Figure 2. Monthly gross precipitation recorded in the experimental area.

Table 1. Total values of throughfall (T), stemflow (Sf), interception losses (I) and gross precipitation (P) in the experimental area from October/2010 to July/2012.

Species	T		SF		I		P
	mm	%	mm	%	mm	%	mm
Aspidosperma pyrifolium	247.35	21.08	179.22	15.27	746.99	63.65	
Cnidoscolus quercifolius	248.81	21.20	146.46	12.48	778.29	66.32	
Croton blanchetianus	180.88	15.41	287.69	24.51	704.99	60.07	1,173.56
Poincianella bracteosa	150.18	12.80	272.55	23.22	750.83	63.98	
Mimosa tenuiflora	188.69	16.08	216.55	18.45	768.32	65.47	

distribution in the study region with long periods of drought and high rainfall concentrated in a few months. The Caatinga is conditioned by the semi-arid climate, with high rates of potential evapotranspiration during the year and low annual rainfall (300 to 1000 mm), normally limited to 3 to 5 months with large temporal irregularity (Reddy, 1983; Santana, 2005).

The total amount estimated for the throughfall ranged from 12.80 to 21.20% between species during the study period, following the descending order: *C. quercifolius* > *A. pyrifolium* > *M. tenuiflora* > *C. blanchetianus* > *P. bracteosa* (Table 1).

In this study, the same rainfall characteristics and meteorological conditions, the differentiated rainfall distribution among the five species can thus be mostly attributable to their differences in morphological characteristics. *C. quercifolius* has a more open crown (Figure 3a), despite the larger leaf area (Table 2), which facilitates water flow in the throughfall.

The stemflow has lower shares in the total precipitated in *A. pyrifolium* and *C. quercifolius* species, representing 15.25 and 12.48% of the gross precipitation, respectively. These results are above those verified by Izidio et al. (2013) with 5.9% in dense Caatinga, Medeiros et al. (2009) with 6% in dense Caatinga, Moura et al. (2009) with 0.4% in the Atlantic Forest and Oliveira et al. (2008) with 1.7% in the eastern Amazon. Yet, the comparison of results obtained for stemflow with other work is hindered by the variability of methods of obtaining and processing the data.

The values obtained for the interception losses were greater than 60%, higher values when compared to Izidio et al. (2013) in dense Caatinga with 17.9% of the precipitate value. These results were obtained by species which still still exceed those found in other forest formations that are below 30% of total rainfall, as Lima and Nicolielo (1983) with 12% in tropical pine forests and 27% for the cerrado, and Franken et al. (1982) with 19.8% in the Amazon forest of solid ground type. On the other hand, Thomaz (2005) found 52.4% loss by capoeira interception, being the closest to the results reported in the present study. The higher evaporation rates, characteristic of the study region, as well as the predominance of events of low precipitation can cause more intercepted rainfall water back to air and thus reduced throughfall through the canopy.

The results found for *C. blanchetianus* indicate a greater efficiency of the stemflow production and a smaller loss of interception than the other species, which has a significant competitive advantage to face periods of water deficit, characteristic of the region.

The results of throughfall, stemflow and interception

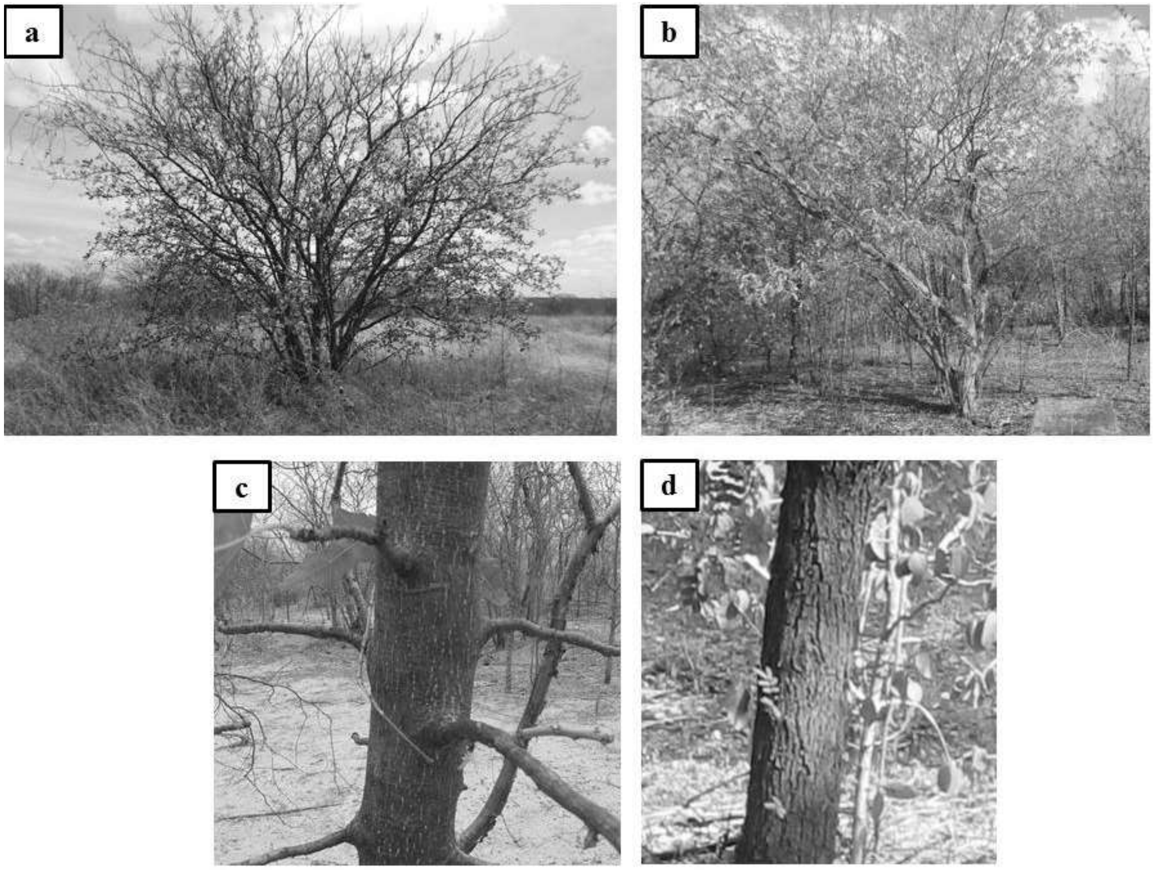

Figure 3. (a) Canopy of *Cnidoscolus quercifolius*, (b) Canopy of *Poincianella bracteosa*, (c) Bole of *Cnidoscolus quercifolius,* and (d) Bole of *Croton blanchetianus.*

Table 2. Average values of leaf area, canopy projection area (CPA), diameter at chest level (DCL) and basal area of the species studied.

Species	Leaf area (cm²)	CPA (m²)	DCL (cm)	Basal area (cm²)
Aspidosperma pyrifolium	6.72	8.90	5.25	21.66
Cnidoscolus quercifolius	31.14	5.23	5.62	24.84
Croton blanchetianus	8.14	6.29	4.40	15.23
Poincianella bracteosa	21.70	7.88	7.53	44.57
Mimosa tenuiflora	1.60	13.84	6.90	37.36

losses presented in this study, regardless of the species, differ in the proportion cited by Bruijnzeel (1990) in tropical forests, where 75 to 96% of the precipitation becomes throughfall, between 1 and 2% is converted into stemflow and between 4.5 and 24% is intercepted by the canopies of trees.

In studies reported for different forest types, the mean values of throughfall, stemflow and interception losses are discrepant. The throughfall was lower than those found by Fan et al. (2014) in native Banksia woodland, Macinnis-Ng et al. (2014) in lower montane tropical rainforest in Panama and Cao et al. (2008) in *Eucommia ulmoides, Vernicia fordii* and *Pinus massoniana*. The stemflow and interception losses was higher than Oyarzun et al. (2011) in Andean temperate rainforests, Macinnis-Ng et al (2014) in Panama, Aboal et al. (1999) in laurel forest and Carlyle-Moses (2004) in a semi-arid Sierra Madre Oriental matorral community.

The differences in the partition of gross precipitation are consequences of the characteristics of the forest community, such as species composition, height, basal area, canopy cover and rainfall characteristics, such as

Table 3. Pearson correlation coefficient (r) for the variables stemflow (Sf), throughfall (T), interception losses (I), leaf area, canopy projection area (CPA), diameter at chest lefel (DCL) and basal area.

Variable	Sf		T		I	
	R	*p*	r	*p*	r	*p*
Leaf area	-0.03	0.9696	0.70	0.2979	-0.92	0.0767
CPA	0.07	0.9096	-0.69	0.1941	0.74	0.1445
DCL	0.19	0.7565	-	-	-	-
Basal area (g)	0.26	0.6717	-	-	-	-

Table 4. Mean values of throughfall (%) and average gross precipitation.

Species	Classes (mm)		
	<177.97	117.97 - 231.94	>231.94
Poincianella bracteosa	10.13	8.08	32.75
Aspidosperma pyrifolium	15.57	23.55	44.57
Mimosa tenuiflora	11.61	12.12	48.37
Croton blanchetianus	11.17	12.84	43.48
Cnidoscolus quercifolius	17.67	18.69	27.72
Gross precipitation (mm)	39.15	218.08	345.92

intensity, and conditions controlling evaporation area.

These percentages below the average are compared to other studies related to the type of formation of the upper canopy leaves of the Caatinga region analyzed, since each ecosystem has distinct characteristics differentiating the redistribution of rainwater, especially the dendrological and phenological patterns of the species studied. The quantities of water involved in throughfall, in stemflow and interception are variable and depend on factors related to both the vegetation and the climatic conditions in which the forest is inserted (Leopoldo and Conte, 1985; Lima and Nicolielo, 1983).

The *C. quercifolius* species had the highest average of throughfall compared to the others, probably this can be related to the peculiarities of the species in relation to architecture and form of canopy, despite the species presents the highest average of leaf area, the projection area of its canopy was the lowest (Table 2).

Notwithstanding, *P. bracteosa* showed the lowest proportion of throughfall, that is, less water crossed the canopy of this species to reach the soil, which may be related to its higher leaf area (21.70 cm²), which favors the rainwater interception and the denser crown (Figura 3b).

Regarding the interception of rainfall precipitation, the *C. quercifolius* had the highest average, the possible explanation is the large leaf area of the species hindering the rain crossing through the canopy, favoring the evaporation of rainwater. Furthermore, the stemflow of this species was the lowest, influencing the calculation of the loss by interception. This low flow is due to the existing amount of branches along the stem of the species which interferes in the fixation of the collecting gutters, reflecting the obtained data (Figure 3c).

The low value lost by interception of the *C. blanchetianus* is closely related to the greater stemflow (24.51%) observed in the species. Despite the basal area and DCL were smaller in *C. blanchetianus*, a smooth and without ramifications stem favored the dripping to the ground (Figura 2d). In smooth stem species, the volume of flow is greater than in those with rough bark.

In Table 3, it shows the relationship between the variables stemflow, throughfall and loss by interception with the parameters leaf area, canopy projection area, DCL and basal area, one can observe that there was no significant correlation between them.

To Lima (1986), the throughfall varies highly in the same species and between species and requires the use of various gauges below the canopy to minimize this effect. The use of a considerable amount of gauges may facilitate the calculation of the average of the spaces in which occurs the concentration of water droplets, at some points in detriment of others (Regalado and Ritter, 2010).

In May, 2011, throughfall was higher in *M. tenuiflora* with 48.37%. *M. tenuiflora* features open canopy, where there is a predominance of leaflets, which explains the higher throughfall that occurred. Throughfall values are influenced by canopy density and the shape of the canopy of trees, which will determine a higher or lower throughfall (Freitas et al., 2013).

In general, throughfall was higher in the class of higher amount of rain, regardless of the species (Table 4).

In the rains of smaller amplitude, most of the water is retained by the dried leaves and twigs and evaporated to the atmosphere. Yet in the most intense rains, part of the precipitated water is retained in the vegetation mass,

Table 5. Mean values of stemflow (%) and average gross precipitation.

Species	Classes (mm)		
	<177.97	117.97 - 231.94	>231.94
Poincianella bracteosa	24.84	8.74	6.70
Aspidosperma pyrifolium	16.53	4.44	4.99
Mimosa tenuiflora	19.53	8.19	4.90
Croton blanchetianus	26.37	7.26	9.51
Cnidoscolus quercifolius	13.13	4.54	6.11
Gross precipitation (mm)	39.15	218.08	345.92

Table 6. Mean values of interceptation losses (%) and average gross precipitation.

Species	Classes (mm)		
	<177.97	117.97 - 231.94	>231.94
Poincianella bracteosa	65.04	83.18	60.54
Aspidosperma pyrifolium	67.90	72.00	50.44
Mimosa tenuiflora	68.87	79.69	46.73
Croton blanchetianus	62.46	79.90	47.01
Cnidoscolus quercifolius	69.20	76.76	66.17
Gross precipitation (mm)	39.15	218.08	345.92

which is saturated, and the remaining water is then drained, increasing throughfall and stemflow. But these processes depend on the characteristics of the previous rainfall event, such as volume and intensity, the type and density of vegetation and the season (Balbinot et al., 2008; Medeiros et al., 2009; Izidio et al., 2013).

With regard to the monthly variation, it is observed that the water drained by the stem was recorded in all evaluated months, and in June, 2011 the species had the lowest proportions.

The *P. bracteosa* species, *M. tenuiflora* and *C. quercifolius*, had the highest percentages of stemflow in October, 2010, while *A. pyrifolium* and *C. blanchetianus* in March, 2012, when it rained only 4.00 mm.

The stemflow is effective in the reposition of water to the ground, since the friction of the water with the bark of plants, besides being directed close to the roots, which diminishes the superficial runoff, allowing this water to easily infiltrate into the soil, reduces the speed of arrival to the ground. Therefore, the stemflow is especially important during low rainfall occurring during the dry season, providing higher soil moisture close to the plant roots in the Caatinga and the maintenance of photosynthetic rates and other functions in their metabolism.

In Table 5, it is observed that in the precipitations of lower amplitude, the amount of water drained by the stem is higher regardless of species, and the *C. quercifolius* obtained the lowest percentage of the precipitation drained by the stem (13.13%) in this class.

C. blanchetianus had the highest average of water drained by the stem (26.86%) in the smallest class of precipitation, suggesting that the species has a better use of precipitated water during the lower rainfall, typical of the dry period, as well as *P. bracteosa*, that also recorded high value. This feature may reflect a longer time for the occurrence of senescence of leaves by the better use of water by the roots.

The interception losses in the studied species remained high during all months of study, peaking in June, 2011 with values above 92%. *P. bracteosa* obtained a low proportion of intercepted precipitation in October, 2010 (10.74%), compared with the other species. Probably the species was defoliated (dry season) by reducing the contact surface of the precipitated water. Yet *A. pyrifolium* intercepted almost the entire precipitation (99.35%) in June, 2011, when it rained 18.05 mm. This species has large leaf area, when compared with the other species of the study. Moreover, *A. pyrifolium* has dense canopy obstructing the passage of rainwater, favoring its loss by evaporation.

In the precipitations of intermediate amplitude, the amount of water lost by interception is greater, regardless of the species, and the *P. bracteosa* had the highest mean percentage of intercepted precipitation (83.18%) (Table 6). This occurs because in the intermediate class the volume of rain, although high, is retained in the leaves and twigs and the precipitations involved in this class occurred at the beginning of the rainy season (February, 2011), after prolonged drought causing the plant biomass dry up, allowing greater water retention in the very first rains. However, in the class of the highest amplitude of rain, the interception losses are low when it is compared to the other species, because these

Table 7. Regression equations, determination coefficient (R²) and significance (p) by species in the experimental area.

Species	Equation	R² (%)	*p*
	$T = 0.163*P^{0.8496}$	46.28	0.0105
Aspidosperma pyrifolium	$SF = 0.3067*P^{0.8325}$	78.44	0.0001
	$I = -2.0259 + 0.7173P$	78.97	< 0.0001
	$T = -0.60918 + 0.3685P$	38.82	0.0229
Cnidoscolus quercifolius	$SF = 0.1179 \times P^{0.9157}$	68.36	0.0005
	$I = 1.0114 \times P^{0.8503}$	74.20	0.0002
	$T = 0.2789 \times P^{0.7822}$	34.51	0.0347
Croton blanchetianus	$SF = -2.9659 + 3.7853*\ln(P)$	72.11	0.0002
	$I = -1.1238 + 0.6189P$	59.66	0.0539
	$T = 0.245 \times P^{0.7932}$	40.33	0.0196
Poincianella bracteosa	$SF = 0.3136 \times P^{0.8098}$	86.17	<0.00001
	$I = 1.7567 + 0.6249P$	59.40	0.002
	$T = 1.7562 + 0.2184P$	47.36	0.0093
Mimosa tenuiflora	$SF = 0.2159 \times P^{0.7835}$	50.61	0.0064
	$I = 0.516 \times P^{1.0344}$	93.62	<0.00001

precipitations have a higher intensity and occurred in the middle of the rainy season when the parts of the plants were already saturated, favoring the stemflow and the throughfall. When compared with other studies in various forest formations, it is verified that the pattern of loss by interception obtained in this study is not similar, as in most studies the major parts of the water intercepted by vegetation occur in the classes of lower range of rainfall (Thomaz, 2005; Moura et al., 2009; Calux and Thomaz, 2013).

Izidio et al. (2013), studying the rain interception in dense Caatinga, found greater interception losses in the classes with lower range of rainfall, differentiating from the present study. It may be related to numerous factors such as different methodologies, conditions of uneven vegetation, such as the preservation and type of vegetation, the distribution patterns of rainfall in space and time, among others.

The data of gross precipitation and throughfall, of the stemflow and of the loss by interception underwent regression analysis by species, whose results are shown in Table 7. In the species studied, the stemflow showed high relation with the amount of gross precipitation, with the lowest value of the determination coefficient (R^2) of 50.61% in *M. tenuiflora* species.

The throughfall, in turn, showed low relations with the gross precipitation in the study period, with R^2 varying between 34.51 (*C. blanchetianus*) and 47.36% (*M. tenuiflora*). The species *M. tenuiflora, A. pyrifolium* and *C. quercifolius*, in this descending order, obtained the highest relations between the interception losses and gross precipitation with R² equal to 93.62, 78.97 and 74.20%, respectively. While the *P. bracteosa* and *C. blanchetianus* showed determination coefficient below 60%.

Conclusions

The results of this study demonstrate that rainfall distribution differences among the five species evaluated under the same meteorological conditions and rainfall characteristics were not related to the leaf area, CPA, DCL and basal area differences between them. *M. tenuiflora* showed greater throughfall in rain events of greater amplitude. The stemflow was more significant in the dry season, especially in species without bifurcations and smooth bark. *C. blanchetianus* had a higher result for stemflow in the lowest precipitation class, suggesting that this species has a better use of precipitated water during the smaller precipitations, typical of the dry period in the region.

The annual loss by interception was higher in *C. quercifolius*. The water lost by interception represented the largest proportion of gross precipitation in all species. This condition may aggravate the water yield of this dry forest.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Comparison of Grafting Techniques and their Effects on some Growth Parameters of Ten Elite Cocoa Clones (*Theobroma cacao* L.)

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Cocoa productivity in Côte d'Ivoire has been on a downward trend for the last decades due to many factors key among them being the non-availability of quality planting materials. However, some elite clones developed in the country are available and need to be adopted by farmers. This study was conducted in Soubré, Côte d'Ivoire to evaluate the grafting techniques (top grafting and budding) success of 10 elite cocoa clones and their effects on selected growth parameters in the nursery and field. Experimental design was a split plot with grafting technique as main factor and clones as subplot treatment replicated three times. Data were collected on shoot emergence 1 to 5 weeks after grafting (WAG) and grafting success at 10 WAG. Our results showed shoot emergence from top grafting but not on budding 1 WAG. In addition, the number of leaves was high in top grafting (11) compared to budding (5); the same trend was observed in the field. There were no significant differences between both techniques for the grafting success; meanwhile success was 77% and 68% for top grafting and budding, respectively. However, there was better vegetative growth on seedlings grafted using top grafting than budding and this technique seems to be the best to graft cocoa in the nursery.

Key words: Cocoa, top grafting, budding.

INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a neotropical, small, evergreen tree and native to the undergrowth of the Amazon forest (South America) (Wood and Lass, 1985), and belongs to the Malvaceae family. It is cultivated around the world, for its seeds used in the manufacture of chocolate, its derivatives and cosmetics. It is a major source of income for developing countries, especially in Côte d'Ivoire, the world's largest cocoa producer, which grows more than one third of the world's supply. The production of cocoa in Côte d'Ivoire in 2013 to 2014 went over 1.7 million tons (CCC, 2014). Nevertheless, the increase of the cocoa production in Côte d'Ivoire has

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been mainly carried out by increasing acreage, causing the degradation of the natural resources in the remaining tropical forest. In addition, many studies revealed that 20 to 30% of the Ivorian cocoa orchards are old and trees that produce less cocoa over time are more prone to pests and diseases (Aguilar et al., 2005; Kéli and Assiri, 2001).

In order to revitalize the unproductive cocoa trees, cocoa growers need to access the high yielding elite planting materials. The current distribution of cocoa hybrid seeds to farmers is not meeting the ever-increasing demand. Moreover, the use of hybrids is known to produce high degree of genetic variability. In Côte d'Ivoire, 71% of cocoa trees are from seeds collected from unselected planting materials (Pokou, 2008). Irizarry and Rivera (1998) reported that only 2 to 3% of the cocoa trees in a population of high-yielding families account for 60% of the total yield, because of the high heterozygous nature of seedlings.

On the other hand, high yielding cocoa clones known for their genetic stability exist in research stations, but have not yet been released to farmers. Clones are easily propagated through grafting (Mng'omba and du Toit, 2006). One advantage with grafting technique is that new plants are identical to the original plant. Furthermore, the technique can be easily executed by farmers. Grafting has successfully been used for vegetative propagation of species such as mango (Mangifera indica), bush mango (Irvingia gabonensis), Akpi (Ricinodendron heudelotii) and Allanblackia spp. (Mng'omba et al., 2010; Munjuga et al., 2013). Grafting is known to shorten the period between flowering and fruiting. Grafting on mature cocoa trees has been demonstrated to double cocoa yield within two years (ICRAF, 2014). This propagation method can also be performed on young seedlings. Several grafting methods exist; among them are top grafting, budding, whip, and whip and tongue. The choice to be used in any particular plant depends on the grafting success. Budding in cocoa has been used for the multiplication of elite clones and has been shown to be expedient in rehabilitating unproductive cocoa trees (Eskes and Efron, 2006). According to Effendy et al. (2013), budding increases production and also stimulates flowering and fruits in cocoa. The most successful method for Allanblackia parviflora is top grafting while budding is the best method for Allanblackia floribunda (Munjuga et al., 2011).

Based on review of available literature, there are no reports on grafting cocoa clones on seedlings in Côte d'Ivoire. The objective of this study was to compare the grafting techniques (budding and top-grafting) and their effects on some growth parameters.

MATERIALS AND METHODS

Study site

Experiments were conducted at the World Agroforestry Centre (ICRAF) Soubré Station, located in the Nawa Region, South-West of Côte d'Ivoire, 5°47′08″N, 6° 36′30″W, 276 m a.s.l. (Figure 1).

Site characteristics

The mean annual rainfall is 1360 mm and varies from 968 to 1767 mm. The minimum and maximum temperature varies from 23 to 30°C. The soils are classified as Ferralsols and Gleysols, generally acidic (5.5), subject to leaching and chemically poor (De Rouw, 1994). The soil characteristics nearby the experimental site were 5.6 for pH, 1.04% for soil organic carbon, 0.1% for total nitrogen and 7.7 mg kg^{-1} for available phosphorus (Diby et al., 2014). Potassium, magnesium and calcium were 0.13, 1.09, and 3.74 cmol kg^{-1}, respectively. Sand content was 41% while the fine particles (silt + clay) were 57%.

Plant

The cocoa clones were obtained from the Centre National de Recherche Agronomique (CNRA) Research Station in Divo. In the nursery, the rootstocks were raised by sowing seeds of an improved cocoa hybrid in polybags filled with topsoil. Three months after planting, the seedlings were grafted with the elite clones. The seedlings were grown for 5 months in a screen house before transplanting them to the field.

Ten elite cocoa clones coded as C1, C8, C9, C14, C15, C16, C17, C18, C20 and C21 were used as scions. Scions were collected from actively growing trees that had no visible signs of disease or pests. C1, C8 and C9 are introduced from the International Quarantine Center Reading, UK while the others are selected locally. All the clones are characterized by high productivity and tolerance to black pod disease.

Experimental design and cultural practices

The experiment in the nursery was a split plot design with two factors and three replications. Grafting method (budding or top grafting) was the main plot treatment and the 10 clones were the subplot treatment. The experimental unit consisted of ten grafted seedlings for each clone giving a total of 600 seedlings for the entire experiments. The grafted seedlings were watered every second day and sprayed with a foliar fertilizer (10 N + 8 P + 10 K + 0.29 MgO) fortnightly.

The experimental design in the field was also a split plot design with three replications, the grafting technique as the main plot treatment and the clones as the subplot treatment. The experimental unit consisted of 5 plants per clone giving a total of 300 plants. Plantain banana seedlings were planted at 2m x 2m to provide temporary shade six months prior to transplanting the cocoa seedlings at the same density as the banana. The seedlings were treated with an insecticide (Deltamethrin and Imidacloprid). A fertilizer (15.5 N + 26.5 CaO + 0.2 B) was also applied at the rate of 100 g/plant once a month. Weeding was carried out on a need basis.

Data collection

In the nursery, the number of grafts with buds (shoots) emerging, leaves and primary branches, the main branch length, the main branch diameter and the number of internodes were recorded every week for 5 weeks. In the field, the number of leaves, primary and secondary branches and the length of the main branch were recorded three months after planting.

The grafting success rate was calculated using the equation as

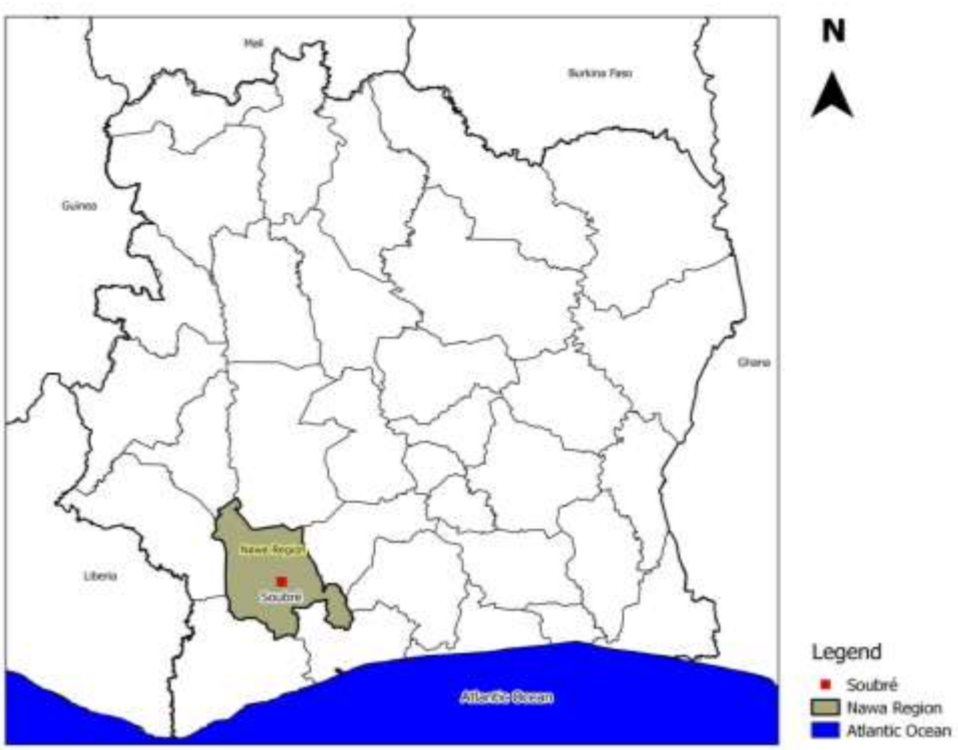

Figure 1. Study location.

follows:

$$\text{Grafting success} = \frac{\text{Number of successful grafts}}{\text{Total number of grafts}} \times 100$$

Data analysis

The data were subjected to two ways analysis of variance (ANOVA), using Statistical Analysis System (SAS) software (SAS, 2003). The differences between means were assessed with the Student-Newman-Keuls (SNK) method at 5%.

RESULTS AND DISCUSSION

Effect of grafting technique on selected growth parameters in the nursery

Shoot emergence: Grafting techniques significantly affected the number of shoots during 1 to 4 week after grafting (Table 1). The differences in shoot formation in relation to grafting technique might be due to the nutritive and nutritional factors like the quantity of carbohydrate stock in the seedling (Tchoundjeu et al., 2010). Budding showed no shoot in all clones compared to top grafting which recorded significant shoots during the first WAG. The lower shoot formation with the budding technique could be attributed to the low carbohydrate reserves (Akinnifesi et al., 2008; Tchoundjeu et al., 2010). During the second WAG, top grafting recorded significantly higher shoots than budding in clones C9, C14 and C16. Top grafting exhibited high and significant number of shoots than budding in clones C16, C17, C18 and C21 three WAG. Top grafting produced more significant number of shoots than budding in clones C16 and C17 while there was no significant difference observed 5 WAG among the grafting techniques. This could be explained by the fact that seedlings that have been top grafted have the ability to renew or to regenerate more easily their cells and have more stored carbohydrates and hormones, because of fresh tissues (Tchoundjeu et al., 2010). Another possible explanation could be that there are a lot of meristematic tissues with high cell division activity at the point of grafting. Before shoot formation, the seedlings rely on the reserve of carbohydrates in their stem tissues. It is also established that the grafting success depends on proper alignment of parenchymatous tissues of both scions and rootstocks (Mohamed et al., 2014; Munjuga et al., 2013) and the skills of grafters (Mng'omba et al., 2010).

Grafting success: There were no significant differences between the two grafting techniques used. This could be probably due to the skills of the grafters or climatic conditions (Munjuga et al., 2013; Ofori et al., 2008). Top grafting recorded 77% grafting success compared to budding which showed a success of 68% (Figure 2). Our finding concurs with work reported by Eskes and Efron

Table 1. Number of shoots emerging one to five weeks after grafting (WAG) according to the grafting techniques

Clones	1 WAG		2 WAG		3 WAG		4 WAG		5 WAG	
	Bud	**T. graft.**	**Bud**	**T. graft.**	**Bud**	**T. graft.**	**Bud**	**T. graft.**	**Bud**	**T. graft.**
C1	0.00^{a*}	0.00^{a}	0.33^{a}	1.00^{a}	6.33^{a}	4.67^{a}	6.33^{a}	5.67^{a}	7.00^{a}	6.67^{a}
C8	0.00^{a}	0.00^{a}	0.67^{a}	1.00^{a}	3.67^{a}	4.33^{a}	4.00^{a}	6.00^{a}	6.67^{a}	6.67^{a}
C9	0.00^{a}	1.33^{a}	1.00^{b}	8.00^{a}	6.67^{a}	8.67^{a}	7.33^{a}	8.67^{a}	8.67^{a}	8.67^{a}
C14	0.00^{a}	0.00^{a}	0.00^{b}	3.33^{a}	3.67^{a}	7.00^{a}	5.33^{a}	8.33^{a}	7.33^{a}	9.00^{a}
C15	0.00^{a}	0.00^{a}	0.67^{a}	1.00^{a}	4.00^{a}	4.00^{a}	5.33^{a}	5.00^{a}	6.33^{a}	6.67^{a}
C16	0.00^{a}	0.00^{a}	0.00^{b}	3.33^{a}	1.00^{b}	6.00^{a}	2.67^{b}	6.33^{a}	4.67^{a}	6.00^{a}
C17	0.00^{a}	0.00^{a}	0.00^{a}	0.33^{a}	2.33^{b}	6.00^{a}	3.67^{b}	9.00^{a}	6.33^{a}	9.00^{a}
C18	0.00^{a}	0.67^{a}	0.33^{a}	3.67^{a}	2.33^{b}	8.67^{a}	4.67^{a}	9.33^{a}	6.33^{a}	9.67^{a}
C20	0.00^{a}	0.33^{a}	0.33^{a}	2.67^{a}	4.67^{a}	6.00^{a}	6.33^{a}	7.00^{a}	7.00^{a}	9.00^{a}
C21	0.00^{a}	1.00^{a}	2.00^{a}	5.33^{a}	4.67^{b}	8.33^{a}	5.00^{a}	7.67^{a}	5.67^{a}	8.33^{a}
Means	0.00^{b}	0.33^{a}	0.53^{b}	2.97^{a}	3.93^{b}	6.37^{a}	5.07^{b}	7.30^{a}	6.80^{a}	7.77^{a}
CV	-	-	-	36.92	59.44	27.51	49.01	25.26	36.81	21.54

*Means followed by the same letter in the same column are not significantly different at the 5% level (SNK). Bud = Budding / T. graft. = Top grafting

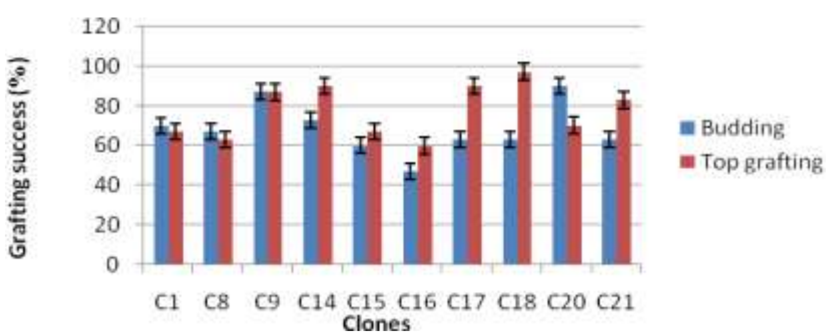

Figure 2. Percent grafting success using different techniques.

(2006) on cocoa who observed that budding technique showed low grafting success rate in Cameroon, Nigeria, and Venezuela. The grafting success was relatively similar using both techniques for clones C1 and C9. With budding, the grafting success was relatively high with clones C9 and C20 and low with clone C16. When top grafting was used, all clones showed a high grafting success percentage compared to budding except for clones C20, C1 and C8. The overall grafting success in nursery in this study (73%) was similar to that in Malaysia (74%) as reported by Figueroa et al. (1991). However, it was low than that observed by Prawoto (2007) who reported high survival rate of grafted cocoa in Indonesia (80%). Consequently, according to these authors, the use of top grafting has been adopted in many countries in order to obtain high success rate. This method is suited to establish clone observation plots and may be applied in future by farmers to multiply preferred clones on their own farm. One disadvantage for using top grafting compared to budding technique is that there are high chances of sprouting from the rootstock, but this is can be addressed by regular pruning.

Growth parameters: Grafting techniques significantly affected selected growth parameters in the nursery (Table 2). The growth pattern observed in the current study using the two grafting techniques are similar to those obtained by Sabir and Kara (2010), Celik (2000) and Alley et al. (1980) while working on grapevines. Top grafting showed the highest number of leaves, primary branches and internodes than budding in all ten clones. It was observed that clones C15 and C1 had good vegetative development with longer internodes. These clones would be good candidates for production of budwood in the clonal garden (Eskes and Efron, 2006). The main branch length was significantly different between the two grafting techniques. Top grafting produced the highest main branch length while, budding showed lowest value in clones C1, C16, C18 and C20. For the main branch diameter, top grafting technique that

Table 2. Effect of grafting technique on selected growth parameters in the nursery.

Clones	Number of leaves		Number of primary branches		Main branch length (cm)		Main branch diameter (cm)		Number of Internodes	
	Bud	T. graft.	Bud	T. graft.	Bud	T. graft.	Bud	T. graft.	Bud	T. graft.
C1	6.81[b*]	13.55[a]	1.00[b]	2.30[a]	12.70[b]	16.14[a]	2.87[b]	3.40[a]	6.81[b]	13.55[a]
C8	4.50[b]	8.30[a]	0.75[b]	2.15[a]	6.79[a]	8.31[a]	1.72[a]	2.46[a]	4.50[b]	8.30[a]
C9	4.92[b]	12.84[a]	0.88[b]	2.36[a]	9.02[a]	11.26[a]	2.13[b]	2.96[a]	4.92[b]	12.84[a]
C14	5.05[b]	10.30[a]	0.82[b]	2.04[a]	8.65[a]	9.77[a]	1.97[b]	2.72[a]	5.05[b]	10.30[a]
C15	5.74[b]	13.75[a]	1.00[b]	2.65[a]	10.13[a]	9.34[a]	2.37[a]	2.27[a]	5.74[b]	13.75[a]
C16	2.50[b]	12.96[a]	0.50[b]	2.28[a]	4.64[b]	11.98[a]	1.11[b]	2.94[a]	2.50[b]	12.94[a]
C17	3.68[b]	8.48[a]	0.74[b]	1.93[a]	6.15[a]	8.67[a]	1.34[a]	2.14[a]	3.68[b]	8.48[a]
C18	4.00[b]	9.69[a]	0.89[b]	2.28[a]	6.33[b]	9.61[a]	1.43[b]	2.73[a]	4.00[b]	9.69[a]
C20	3.70[b]	10.62[a]	0.81[b]	2.00[a]	4.93[b]	10.56[a]	1.13[b]	3.18[a]	3.70[b]	10.62[a]
C21	5.65[b]	11.68[a]	0.88[b]	2.16[a]	9.24[a]	11.10[a]	2.06[a]	2.62[a]	5.65[b]	11.68[a]
Means	4.70[b]	11.07[a]	0.84[b]	2.20[a]	7.9[b]	10.55[a]	1.82[b]	2.72[a]	4.70[b]	11.07[a]
CV	53.7	49.54	44.08	39.8	63.66	45.74	71.62	45.21	53.7	49.54

*Means followed by the same letter in the same column are not significantly different at the 5% level (SNK). Bud = Budding / T. graft. = Top grafting

Table 3. Effect of grafting technique on selected growth parameters of cocoa clones in field.

Clones	Number of leaves		Number of secondary branches		Number of primary branches		Main branch length (cm)	
	Bud.	T. graft.	Bud.	T. graft.	Bud.	T. graft.	Bud.	T. graft.
C1	34.33[b*]	55.80[a]	5.73[b]	12.40[a]	1.20[b]	2.20[a]	55.73[a]	54.93[a]
C8	27.07[a]	33.20[a]	5.47[a]	5.13[a]	0.93[b]	2.00[a]	35.73[a]	37.60[a]
C9	35.07[a]	43.00[a]	6.13[a]	7.27[a]	1.27[b]	2.40[a]	47.20[a]	45.63[a]
C14	47.40[a]	37.80[a]	8.67[a]	8.00[a]	1.00[b]	2.07[a]	48.30[a]	35.47[b]
C15	40.00[a]	56.87[a]	9.33[a]	13.40[a]	1.13[b]	2.67[a]	43.47[a]	42.27[a]
C16	17.20[b]	50.53[a]	3.67[b]	11.60[a]	0.67[b]	1.93[a]	20.47[b]	41.60[a]
C17	24.07[b]	51.60[a]	3.80[a]	4.93[a]	0.73[b]	2.07[a]	30.60[b]	51.13[a]
C18	28.80[a]	34.87[a]	4.80[a]	5.00[a]	1.20[b]	2.00[a]	36.00[a]	34.80[a]
C20	25.20[b]	51.47[a]	4.00[b]	9.73[a]	0.93[b]	1.80[a]	43.67[a]	47.40[a]
C21	36.27[a]	45.60[a]	5.80[a]	7.20[a]	1.00[b]	1.73[a]	44.93[a]	42.40[a]
Means	31.54[b]	46.07[a]	5.74[b]	8.47[a]	1.01[b]	2.09[a]	40.61[a]	43.32[a]
CV	63.19	51.45	70.48	61.21	64.81	49.85	47.01	36.86

*Means followed by the same letter in the same column are not significantly different at the 5% level (SNK). Bud = Budding / T. graft. = Top grafting

promoted shoot development yielded the thickest main branch compared to budding technique in clones C1, C9, C14, C16, C18 and C20. In the overall study, top grafting technique produced higher values for all parameters than budding technique in nursery. Indeed, top grafting produced double the number of leaves, primary branches and internodes and exhibited more main branch length and large branch diameter than budding. These results concur with those of Akinnifesi et al. (2008) and Tchoundjeu et al. (2010).

Effect of grafting technique on selected growth parameters in the field

The results of the present study showed that top-grafting yielded the best results in most cases. Top grafting exhibited the highest number of leaves than budding in cocoa clones C1, C16, C17 and C20 (Table 3). The number of secondary branches was higher in top grafting compared to budding in clones C1, C16 and C20. Top grafting produced more elongation of the shoots than budding in clones C16 and C17. The number of leaves, secondary branches and primary branches contribute to the vegetative development of cocoa clones, and thus, contribute to higher yield (Salehi-Mohammadi et al., 2009). Unlike the other clones, the main branch was longer using budding technique compared to top grafting. Top grafting produced double the number of primary branches and more number of leaves and secondary branches than budding in the field. These results are similar to those of Sabir and Kara (2010) and Eskes and Efron (2006). During the current study, grafting techniques were found to have some effect on selected growth parameters of cocoa clones in field. These findings contradicts those of Effendy et al. (2013) who reported that side grafting is by far the most popular grafting method in cocoa in Indonesia. However they concur with Effendy et al. (2013) as some clones like C20, C1 and C8 exhibited high grafting success using budding compared to top grafting. These results are similar with those of Tesfaye et al. (2014) who found that in coffee, the grafting techniques influences vegetative growth parameters and can be used as a criterion to screen precocity materials. Most clones exhibited high grafting success when top grafting was used and it was proposed therefore in this study that this should be adopted for grafting the elite cocoa clones in the study areas.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Gas exchange and chlorophyll content in tomato grown under different organic fertilizers and biofertilizer doses

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The correct management of organic fertilization has been shown as an extremely viable alternative in the production of vegetables, providing high yields concomitant to the reduction of synthetic fertilizers. The improvement of the production can be interpreted by the physiological behavior, favored by the organic fertilization with the supply of nutrients. Thus, an experiment was carried out to evaluate the gas exchange and Soil-Plant Analyses Development (SPAD) chlorophyll content in tomato plants according to types of organic fertilizers and biofertilizer doses. The experimental design was completely randomized with treatments distributed in factorial arrangement (3 x 5), referring to organic fertilizer types (T1: earthworm humus; T2: goat manure and T3: cattle manure) and biofertilizer concentrations (600, 800, 1000, 1200 and 1400 ml), with four replications. The gas exchange and the SPAD chlorophyll content in tomato plants depend on the type of organic fertilizer and the concentration of biofertilizer. It was possible to observe that increasing doses of biofertilizer in the substrate with low organic concentration increase the gas exchange in tomato plants, while high doses together with more concentrated organic fertilizers reduce these characteristics.

Key words: Alternative fertilizer, *Lycopersicon esculentum*, photosynthesis, organic fertilizer.

INTRODUCTION

The tomato (*Lycopersicon esculentum* Mill.) is a plant of great economic importance, and is acknowledged to be among the most consumed vegetables, due to the high nutritional value of its fruits, rich in antioxidants (Filgueira, 2008). The fruit yield and quality are influenced by several factors, such as growth conditions, which include plant nutrition (Oliveira et al., 2014; França et al., 2017; Ersahin et al., 2017).

Among the tomato cropping systems, organic fertilization needs to be highlighted because it improves the soil and the plants, resulting in production reductions, since the producer can replace the commercial substrate with inputs found on the site (Oliveira et al., 2013a). In addition, the demand for organic products has expanded in recent times, opening possibilities of adding value to products (Santos et al., 2013).

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The tomato crop responds satisfactorily to organic fertilization, however, factors such as type and quantity of fertilizers are determinant in improving plant growth and adequate development (Mueller et al., 2013, França et al., 2017).

The types of fertilizers seem to vary greatly in their influence on plant growth, probably due to the composition and, consequently, to the number of nutrients offered (Oliveira et al., 2014). However, different organic compounds such as earthworm humus, goat manure and cattle have positively influenced the plants when used in an adequate amount. The worm humus can be an excellent alternative to increase the commercial substrate in the organic production system, since mineral fertilizers are not permitted (Oliveira et al., 2013a). Already, it is known that the goat manure has better structure than other types, allowing better aeration, and therefore it ferments quickly and can be availed after a less period of decomposition (Cavalcante et al., 2010), while the cattle manure brings benefit to the plants, because it provides organic nitrogen accumulation in the soil (Oliveira et al., 2014; Santana et al., 2017). In addition, the combined use of organic fertilizer and biofertilizer can be a good alternative for organic fertilization in tomato, because besides providing more quantity may promote more displacement of nutrients to the roots.

The response of organic fertilization has been positively verified as a viable alternative in the production of different vegetables, such as tomato (Yanar et al., 2011; Mueller et al., 2013; França et al., 2017), okra (Oliveira et al., 2013b; Gomes et al., 2017), sugar beet (Gondim et al., 2015), eggplant (Santos et al., 2013) and gherkin (Oliveira et al., 2014).

However, although the effects of organic fertilizer on the growth and production of several vegetable species have been widely reported, studies evaluating the physiological responses as gas exchange and chlorophyll content are not common. Some researchers investigated the gas exchange in tomato plants cultivated under drought stress (Zhu et al., 2012) and salinity (Horchani et al., 2010), which observed changes as to the cultivation conditions, demonstrating the importance of evaluating also under organic cultivation. Photosynthesis is the main physiological process that is affected by changes in growth conditions, can be evaluated by gas exchange measurements based on CO_2 assimilation, as well as chlorophyll content, based on the SPAD index (Santos et al., 2010; Zhu et al., 2012). Towards the exposed, the objective of this work was to evaluate the influence of different types of organic fertilizers and doses of biofertilizer in the gas exchange and SPAD chlorophyll index in tomato plants.

MATERIALS AND METHODS

The experiment was conducted between July and September 2016, in a protected environment, in the State University of Paraíba, Campus IV, Paraíba, Brazil. The site is situated in the coordinates 6°20'38" S 37°44'48' W and 275 m of altitude. The climate of the region according to the classification of Koppen is of type BSWh, that is, hot and dry, with two distinct seasons: one rainy with irregular precipitation and another without precipitation. The maximum, average and minimum internal temperature in the protected environment was around 42, 34 and 19°C, respectively, with relative air humidity varying from 35 to 52% between the months of conduction of the experiment. The design was completely randomized, with factorial arrangement 3 × 5, referring to three types of fertilizer: T1 = 20% earthworm humus + 80% soil; T2 = 30% goat manure + 70% soil; T3 = 40% cattle manure + 60% soil, and five biofertilizer doses: 600, 800, 1000, 1200 and 1400 ml, with 4 replications, totaling 60 experimental units. Doses of biofertilizer were split and applied two times, the first being the 25 DAT (days after transplanting), and the second at 35 DAT. For the substrate composition, was used a eutrophic Flubic Neosol (EMBRAPA, 2013), plus percentages of earthworm humus, goat manure and cattle. After collecting soil samples in the superficial layer (0-20 cm), the chemical and physical characteristics were determined (Table 1). The chemical characteristics of earthworm humus and goat and cattle manure were also determined (Table 2), according to the methodology proposed by EMBRAPA (2011). The preparation of the bovine biofertilizer was carried out according to Santos et al. (2014), lasting approximately 35 days for complete fermentation, and obtaining the liquid compound (Table 3).

The seeds were sown in trays containing 128 cells, adding three seeds per cell filled with commercial substrate Basaplant®. The thinning was performed when the seedlings presented a definitive pair of leaves, approximately 10 days after sowing (DAS), leaving the most vigorous. The seedlings were irrigated daily until transplanting, which was carried out in plastic pots filled with 7 kg of soil + substrate corresponding to each treatment. The tomato variety used was I-5300 (cv. Santa Clara), widespread in Brazil, whose seed germination was 96% and purity 99%. The irrigation of the plants was performed with a uniform volume of water, as a function of crop evapotranspiration. The volume applied (Va) per container was obtained by the difference between the average weight of the container in the condition of 100% of the available water (Paw) and the average weight of the containers in the current condition before irrigation. The weight of the pots with soil field capacity (100% water available) was determined by saturating the soil and submitting to drainage; when the volume was decreasing, the pots were weighed and the difference in weight in relation to the vessels (Paw) was considered as evapotranspirated water, whose volume was restored.

At 45 days after transplanting (DAT) measurements of the gas exchange were made on the third leaf from the apex, with the help of the portable infrared carbon analyzer (IRGA), model LCPro+ Portable Photosynthesis System® (ADC BioScientific Limited, UK), with temperature adjusted to 25 °C, irradiation of 1800 μmol photons m^{-2} $s^{-1}$and flow of air 200 ml min^{-1}. The physiological variables evaluated were internal CO_2 concentration (Ci - μmol mol^{-1}), stomatal conductance (gs - mol H_2O m^{-2} s^{-1}), transpiration (E - mmol H_2O m^{-2} s^{-1}) and photosynthesis (A - μmol CO_2 m^{-2} s^{-1}). The instantaneous efficiency of water use (WUE) was obtained from the relationship between photosynthesis rate (A) and transpiration (E), and the instantaneous efficiency of carboxylation (EiC) between photosynthesis rate (A) and the internal concentration of carbon (Ci).

The SPAD index readings were performed on the same leaf used in the gas exchanges, using the SPAD-502 Chlorophyll Meter. Three readings per plant were collected, aiming at greater representativeness. Then, the average per plant was calculated, on the equipment itself. Data were submitted to analysis of variance, at a significance level of 5% probability. When significant, the regression analysis was performed for the unfolding of the s

Table 1. Chemical and physical characteristics of the soil used in the experiment.

Chemical	Values	Physical	Values
Hydrogen ion potential (H_2O) (1:2.5)	6.7	Sand (g kg^{-1})	640.00
Calcium ($cmol_c dm^{-3}$)	1.49	Silt (g kg^{-1})	206.00
Magnesium ($cmol_c$ dm^{-3})	0.54	Clay (g kg^{-1})	154.00
Sodium ($cmol_c dm^{-3}$)	0.10	Textural classification	Sandy frank
Potassium ($cmol_c dm^{-3}$)	1.72	Total Density (g dm^{-3})	1.54
Sum of bases ($cmol_c dm^{-3}$)	3.85	Density of particles (g dm^{-3})	2.68
Hydrogen + Aluminum ($cmol_c dm^{-3}$)	0.00	Total porosity (%)	42.54
Cation exchange of capacity ($cmol_c dm^{-3}$)	3.85	Field capacity (g kg^{-1})	146.9
Bases Saturation (V %)	100	Permanent wilting point (g kg^{-1})	76.60
Qualitative calcium carbonate	Wanting	Water available (g kg^{-1})	70.3
Organic carbon (%)	0.67		
Organic matter (%)	1.2		
Nitrogen (%)	0.07		
Assimilable phosphorus (mg dm^{-3})	16.83		

Table 2. Chemical characteristics of the organic fertilizers used: earthworm humus, goat manure and bovine.

Mineral nutrients												
N	P	K	Ca	Mg	Na	Zn	Cu	Fe	Mn	MO	CO	C/N
.... g kg^{-1}				.... mg kg^{-1}						.... g kg^{-1}		
Earthworm húmus												
11.8	0.4	4.1	14.2	4.0	-	84	10.8	-	237	-	-	-
Goat manure												
21.9	5.0	3.10	38.2	4.5	7.0	55	33	9567	370	433.0	340.5	15:1
Cattle manure												
12.7	2.5	16.7	15.5	4.0	5.59	60	22	8550	325	396.0	229.7	18:1

MO: organic matter; CO: organic carbon; C/N: carbon nitrogen ratio.

Table 3. Chemical characteristics of the biofertilizer used in the experiment.

Chemical characteristics	Values
Hydrogen ion potential	7.10
Electric conductivity - dS m^{-1}	5.13
Cations - $cmol_c$ L^{-1}	---
Calcium	1.75
Magnesium	1.20
Sodium	1.34
Potassium	0.91
Anions - $cmol_c$ L^{-1}	---
Chloride	2.53
Carbonate	0.33
Bicarbonate	1.56
Sulfate	0.79

biofertilizer doses, and the Tukey test for comparison of the substrates, using SISVAR.

RESULTS AND DISCUSSION

There was a significant effect of the interaction between types of fertilizer and doses of biofertilizer for stomatal conductance, transpiration, photosynthesis and instantaneous carboxylation efficiency (Table 4). As for the isolated factors, types of fertilizer affected stomatal conductance, internal CO_2 concentration and SPAD, while only the internal CO_2 concentration had no affected by the biofertilizer dose. Stomatal conductance increased by 275% in T1 fertilizer, with the increase of biofertilizer doses until 1400 ml. In T2 and T3, gs reached a maximum of 0.066 and 0.103 mol H_2O m^{-2} s^{-1} at the estimated doses of 1100 and 1225 ml $plant^{-1}$, respectively (Figure 1A). Similarly, transpiration increased 56.7% in the T1 fertilizer, with the increase of biofertilizer doses until 1400 mL, while in T2 and T3 transpiration reached a

Table 4. Summary of variance analysis (values of F) for stomatal conductance (gs), transpiration (E), photosynthetic rate (A), internal CO_2 concentration (Ci), water use efficiency (WUE), instantaneous carboxylation efficiency (EiC), foliar temperature (Tleaf), chlorophyll content (SPAD) and root-shoot ratio (R-SR) in tomato plants grown under different organic fertilizers and biofertilizer doses.

Sources of variation	gs	E	A	Ci	WUE	EiC	Tleaf	SPAD	R-SR
Types of Fertilizer (T)	3.17*	2.32^{ns}	0.49^{ns}	6.37**	2.44^{ns}	2.13^{ns}	0.09^{ns}	16.73**	61.83**
Biofertilizer doses (D)	15.51**	15.95**	14.97**	1.34^{ns}	2.58*	12.93**	9.56**	9.70**	11.00**
Interaction (TxD)	4.74**	4.00**	2.87*	0.96^{ns}	0.77^{ns}	2.60*	0.57^{ns}	1.58^{ns}	28.96**

* and ** Significance level of 5 and 1%, respectively, whereas, ns no statistical differences.

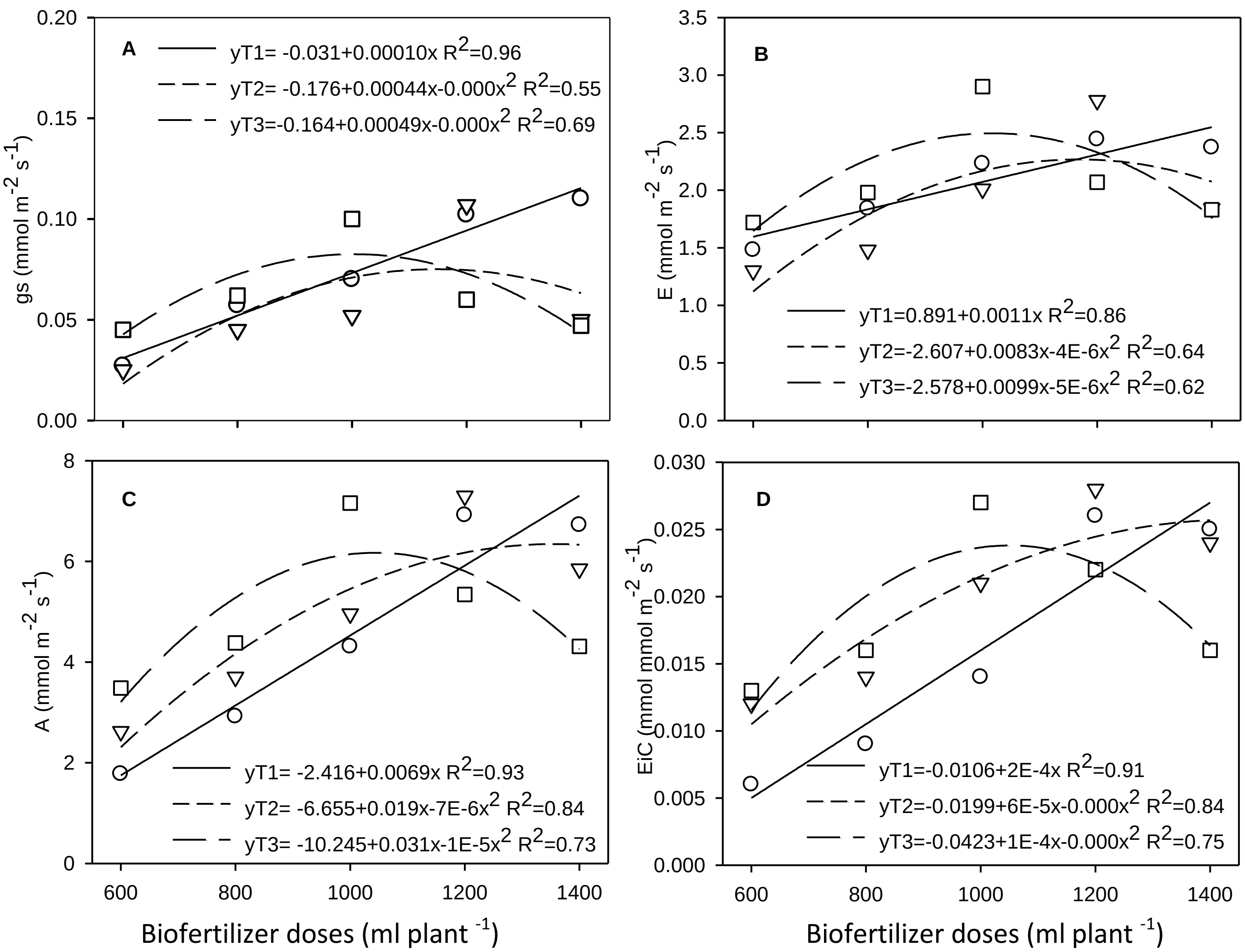

Figure 1. Stomatal conductance - gs (A), transpiration - E (B), photosynthetic rate - A (C) and instantaneous carboxylation efficiency - EiC (D) in tomato plants grown with different organic fertilizers and biofertilizer doses.

maximum of 1.70 and 2.32 mmol H_2O m^{-2} s^{-1} at the estimated doses of 1037.5 and 990 mL $plant^{-1}$, respectively (Figure 1B).

The photosynthetic rate also increased linearly in T1, reaching 320% with the addition of biofertilizer doses until 1400 ml $plant^{-1}$, while in T2 and T3 the photosynthesis reached the maximum of 6.23 and 6.25 μmol CO_2 m^{-2} s^{-1}, with the estimated doses of 1357 and 1049 ml $plant^{-1}$, respectively (Figure 1C). Likewise, EiC increased by approximately 380% with increases in biofertilizer doses in T1, while in T2 and T3 the maximum EiC of 0.023 and 0.017 μmol m^{-2} s^{-1} was reached in the estimated doses of 1250 and 1000 ml $plant^{-1}$, respectively (Figure 1D).

The stomatal conductance, transpiration, photosynthesis and carboxylation efficiency had the same behavior in each type of fertilizer, indicating that both water loss and carbon fixation were influenced by stomatal control, as observed in tomato under drought stress (Morales et al., 2015). In the T1 fertilizer, the linear growth of these characteristics with the increase of the biofertilizer doses indicates that the tomato plants cultivated with 20% earthworm humus use the incorporation of biofertilizer until 1400 mL $plant^{-1}$, unlike T2 and T3 that decreased the gas exchange of the tomato with the increase of the doses of biofertilizer. These results probably occurred due to the concentration of the types of fertilizers, because as in T1 the proportion of soil was higher, the increase of the doses of biofertilizer favored in the increase of the gas exchanges by supplying nutrients in a gradual way, complementing the hummus. It was observed in tomato plants under different organic fertilizers, that in the treatments with 25% earthworm humus, the plants had lower height and root length, compared to treatments composed of 50 and 100% humus (Oliveira et al., 2013a), demonstrating that the low amount of this organic fertilizer is insufficient in the nutrition of the tomato plants and, therefore the doses of biofertilizer were useful in the mineral supplementation of the substrate.

On the other hand, in the substrates with goat manure and cattle, the highest doses of biofertilizer were more than necessary for tomato plants, as observed by reductions of the gas exchanges at the highest applied dose. This could possibly be due to nutrient supply in addition to that the plants especially needed macronutrients, as occurred in okra plants in which cattle manure increased foliar N, P and K when associated to the application of the biofertilizer (Oliveira et al., 2014). Also, the increase of the doses of biofertilizer associated with the concentration of manure in both substrates may have compromised the growth of tomato plants. In the watermelon culture, it was observed that the increase in doses (L pit^{-1}) of both goat and cattle manure had a threshold for plant length and diameter (Cavalcante et al., 2010).

The lowest leaf temperature of 34.05 °C was reached with the maximum estimated dose of 1037.4 mL $plant^{-1}$, regardless of the type of fertilizer used (Figure 2A). However, among types, the internal CO_2 concentration was higher in T1, followed by T3 and T2 (Figure 2B).

The low stomatal conductance reduces transpiration, decreasing the cooling capacity of the leaf and increasing its temperature, as observed in plants of all types of fertilizer with the lowest dose of biofertilizer, and in manure treatments associated with a higher dose, which seems to have configured nutritional excess, since the characteristics of gas exchange are useful in the interpretation of physiological changes in the plants when subjected to adverse conditions, such as low and high amount of nutrients (Gondim et al., 2015). In other words, high gs leads to the increase of Ci (Santos et al., 2010), as observed in this study, in which the linear increase of stomatal conductance in T1 fertilizer led to higher internal carbon concentration. The WUE increased approximately 122% with the addition of biofertilizer doses, regardless of the type of fertilizer used (Figure 2C). Already, the SPAD chlorophyll content was higher in the T2 and T3 fertilizers (Figure 2D), while among the biofertilizer doses, the maximum SPAD was 42.6% reached at the maximum estimated dose of 999.5 mL $plant^{-1}$ (Figure 2E).

The increase in the WUE in relationship with the increase in the doses of biofertilizer may be due to the lower transpiration in relation to the photosynthetic rate, or the higher nutrient supply by increasing the doses of biofertilizer (Oliveira et al., 2014), as verified in lettuce plants in which organic fertilization improved water use efficiency (Santos et al., 2010). It was also verified in tomato plants, that organic fertilizers contribute to the adequate growth and development of plants and to the correction of nutritional deficiencies (Dinu et al., 2015; Kalbani et al., 2016).

In a study with lettuce plants, was observed that the SPAD chlorophyll content was not altered by organic fertilizer types, among them the cattle manure (Santos et al., 2010) differing from the results of this work, in which the substrates with goat manure had higher SPAD. Furthermore, the higher dose of biofertilizer decreased the chlorophyll content of tomato plants, a fact that may have led to the reduction of gas exchange. The chlorophyll content was not altered in tomato plants fertilized adequately (Zhu et al., 2012), but reduced in wheat and rice plants with the increase of N supply, configuring excess of this nutrient (Swain and Sandip, 2010; Hasan et al., 2016). This fact probably occurred in this study in tomato plants fertilized with bovine and goat manure together with the highest doses of biofertilizer. The root-shoot ratio increased linearly 27.4% with the addition of biofertilizer doses in the T1 fertilizer, while in T2 and T3 this ratio was maximum of 0.213 and 0.205, respectively, in the estimated dose of 1000 mL $plant^{-1}$ for both types of fertilizer (Figure 2F).

During plant growth, fertilizers present on the substrates, especially T2 and T3, coupled with the supply of the biofertilizer and nutrients contained in the soil, the nutritional requirements of the crop beyond the appropriate, as evidenced by the increase of the root-shoot ratio, besides the gas exchange and the chlorophyll content SPAD. The lower root-shoot ratio is a reflection of the reduction in the dry mass of the roots, as observed with the higher doses of biofertilizer and the fertilizers T2 and T3, which can occur due to the excess of nutrients released with the increase of the organic fertilization, as well as observed in okra plants grown on grape marc substrate and fertilized with slow-release fertilizers (GOMES et al., 2017), or can be attributed to increased electrical conductivity with organic fertilizers (Ersahin et al., 2017).

On the other hand, the reduction of root-shoot ratio

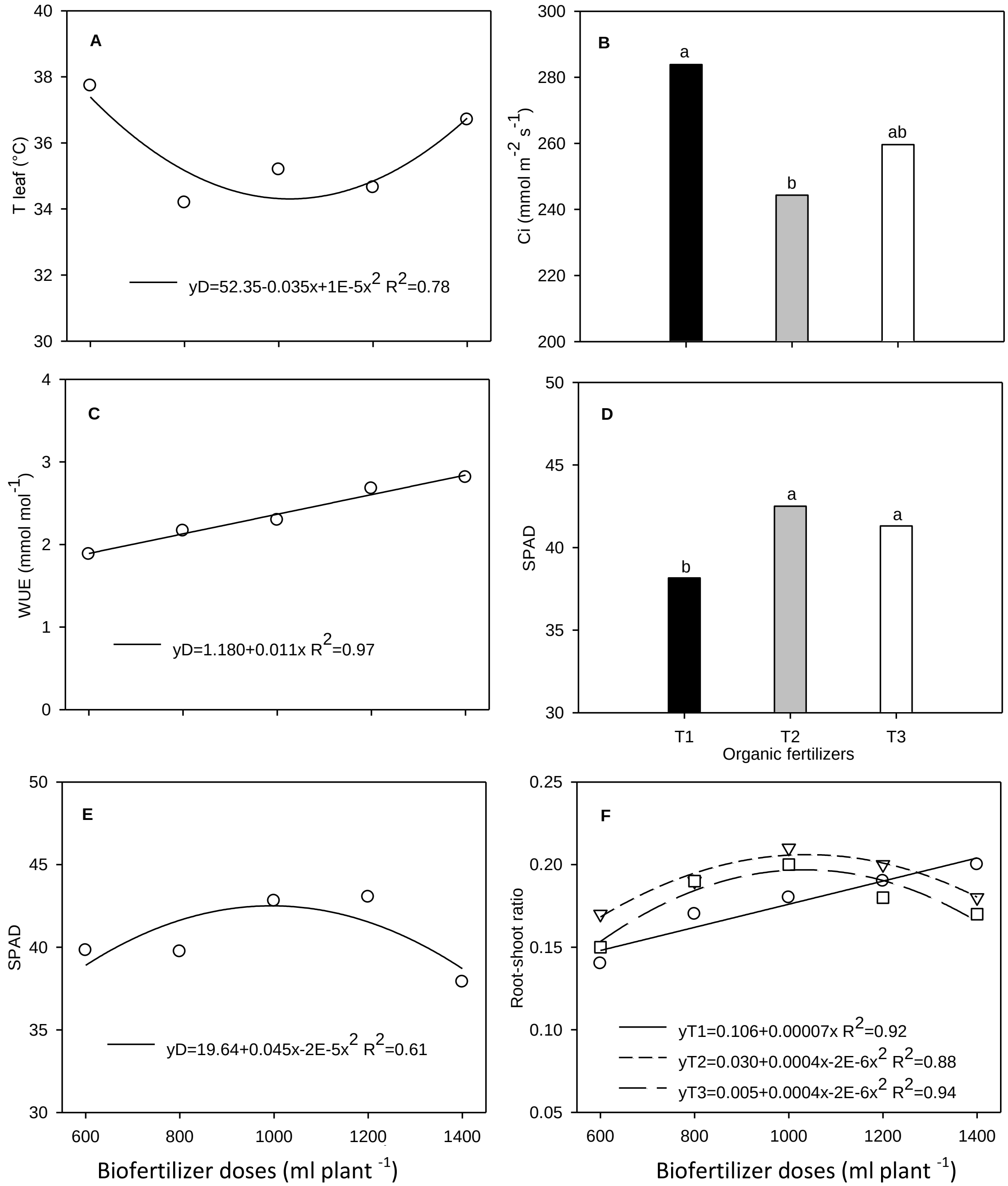

Figure 2. Leaf temperature - Tleaf (A), internal CO_2 concentration - Ci (B), instantaneous water use efficiency - WUE (C), chlorophyll content SPAD (D and E) and root-shoot ratio (F) in tomato plants grown with different organic fertilizers and biofertilizer doses.

observed in plants cultivated on manure substrates and high doses of biofertilizer may mean that these plants, once the needs of the root growth have been satisfied by the greater absorption of the nutrients provided by the biofertilization, have passed; they direct their activities to the formation of the aerial part, such as leaves and fruits (Oliveira et al., 2014; Cruz et al., 2015; Kalbani et al., 2016).

Conclusion

The concentration of organic fertilizer in the soil and the sociation with doses of biofertilizer influence the gas exchange and SPAD chlorophyll content in tomato plants. In addition, increasing doses of biofertilizer with organic fertilizer (T1) increase the gas exchange in tomato plants, while high doses together with more concentrated organic fertilizers (T2 and T3), decreases these characteristics.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Mechanical harvest methods efficiency and its impacts on quality of narrow row cotton

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The large scale cultivation of cotton in the Cerrado areas was made possible by large investments in technology, particularly in mechanized harvesting, which makes the business viable. However, the use of harvesters causes qualitative and quantitative losses to the final product, reducing the profitability of production. This study aimed to evaluate the fiber characteristics of narrow row cotton using harvesting Pro-12 VRS picker and stripper. The experiment was conducted on a farm in the municipalities of Sorriso in the 2014 agricultural season. The experimental design was randomized blocks with seven replicates. The treatments consisted of five harvesting systems: Pro-12 VRS picker, finger stripper with and without field cleaner and brush stripper with and without field cleaner. The efficiency of the harvester was quantified by determining the yield and total loss. Impurities in the harvested cotton were quantified by determining the percentage of bark and stem present in the sample. The following technological fiber characteristics were analyzed through the HVI tool: Trash, UHM, SFC, Elg, Mic, +b, Rd, UI and STR. Cotton in hardened management system has less trash content when harvested with the Pro-12 VRS picker. The Pro-12 VRS picker, however, failed to preserve the intrinsic quality of the fiber.

Key words: Harvester's cotton, platforms picker, fiber cotton characteristics.

INTRODUCTION

Harvesting loss is an important factor for evaluating the performance of a cotton picker. It determines the amount of cotton that is collected from the field, and subsequently cleaned with ginning. Harvesting loss also determines the amount of fiber available for marketing. According to Faulkner et al. (2011), mechanical cotton harvesting increases losses; however, work efficiency gains already far exceed the losses in harvest efficiency.

The cotton stripper has increased harvest efficiency and consequently lower crop losses than harvester spindles (picker) (Faulkner et al., 2011). According to Williford et al. (1994), harvesters will, with time, be able to achieve efficiency of up to 95% but may remain between 85 and 90% efficient.

Boll characteristics and plant height may dramatically affect crop losses. Corley (1966) measured picking

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efficiencies of 95% for fluffy bolls compared to 90 and 65% for weathered and knotty bolls, respectively. The increase in crop losses may be the result of seed cotton, left in the cotton plant or lying on the ground after harvesting (Kepner et al., 1978).

The stripper-harvested cotton contains more foreign matter than cotton harvested with the picker harvester. The greater presence of foreign matter generates higher costs for transport to the cotton bale, as well as the potentially higher cost of processing cotton (Faulkner et al., 2007). However, Faulkner et al. (2011) point out that most stripper harvesters are equipped with seed cotton cleaners, removing about 60% of this material in the field.

Faulkner et al. (2011) demonstrated that the stripper harvester is more efficient than the picker, but the micronaire values, length and uniformity are better with a spindle harvester due to fiber maturity.

According to Mcalister Iii and Rogers (2005), when evaluating the effects of sampling methods on the quality of high-density cotton fiber in the United States, it showed that samples harvested with a picker had better micronaire, fiber strength, average length, length uniformity, yellowness, and decreased neps than samples harvested with a stripper. In another evaluation of sampling methods, Jost and Cothren (2000) studied various row spacings and found no influence of these resistance and fiber fineness. These results highlight the need for a more well-developed understanding of the effects of cotton harvest methods, on the quality of the harvested product.

Therefore, this study aims to present the collection system that provides lower quantitative and qualitative losses of cotton fiber, harvested in the state of Mato Grosso in a dense cultivation system.

MATERIALS AND METHODS

This work was carried out in the municipality of Sorriso, MT (12°35'16'' S, 55°48'27'' W), average altitude of 360 m, in the 2014 agricultural year. The seeding was performed with cotton variety IMA 5672 with spacing of 0.45 m between rows. At the end of the cycle, fifteen days before harvest, was applied defoliant and maturator throughout the experimental area. Experimental design consisted of a randomized block with the following collection system treatments: Pro-12 VRS picker, finger stripper with field cleaner, finger stripper without field cleaner, brush stripper with field cleaner and brush stripper without field cleaner. Each treatment had seven repetitions, totaling 35 experimental plots.

Each plot measured 3.6 m wide by 30 m long, totaling 108 m^2. The carriers used to maneuver the machines were 10 m long. The harvesters were conducted by the same operator throughout the experiment, which kept the average speed of the stripper harvester 0.65 m s^{-1} and Pro-12 VRS picker harvester at an average speed of 1.43 m s^{-1}. The average speed of harvesters was determined by monitoring the time the machines traveled a distance of 50 m. This procedure was performed three times. Thus, speeds were determined by dividing the distance traveled by the average time.

The machines and platforms used in narrow-row cotton harvest were the John Deere model 9930 Pro-12 VRS cotton picker with Agrotech platform for dense cotton with 4 rows spaced 0.45 m apart (Figure 1A); John Deere brush stripper model 9960 with 6-line platform (Figure 1B); John Deere finger stripper harvester model 9960 with an EMA S-0036 platform 3.66 m wide (Figure 1C).

Before harvesting, the agronomic characteristics were quantified within the borderlines with an area of 4.5 m² measured 5.0 × 0.90 m. Monitoring of the fiber moisture occurred before and during the harvest, using a calibrated Hygron portable measuring device. Fiber samples were removed from within the experimental area and the collection was initiated when the humidity was equal to or less than 7%.

Productivity of experimental areas was estimated using a demarcation 4.5 m², within which whole cotton plants were manually collected. Productivity per hectare was determined by weighing the samples. Pre-harvest losses resulting from climate and crop management conditions were collected manually. This process involved, collecting all seed cotton on the ground surface within the demarcation of 4.5 m². In the laboratory, samples of pre-harvest losses were weighed after removal of foreign material present in the fiber. After the passage of the harvester, post-harvest losses were obtained by manual collection of cotton that remained trapped in the plant, as well as cotton that fell on the soil surface, using the demarcation of 4.5 m² in the center of the plot.

The total sum of the weight of cotton found on the soil surface and remaining in the plant comprises a quantitative total loss of the experiment. As such, it is possible to determine the efficiency of the harvester by Equation 1, as noted by Rodriguez (1977).

$$\text{Efficiency of the harvester} = \frac{100 \times \text{harvested cotton}}{\text{harvested cotton} + \text{post harvest losses}} \quad (1)$$

To determine the qualitative losses, samples were taken from inside the basket of the harvester. These samples were collected at the time that the harvester was in the middle of the plot. In the laboratory, to measure the amount of contaminants present in the fiber in these samples, the impurities were separated from the manual form of fiber and classified into two categories (stem and bark) for later weighing.

The basket of the samples was sent to the laboratory, which passed through a ginner of 20 saws for separating the lump of cotton lint. After ginning, down from the sub-samples was sent to the laboratory of UNICOTOON in Primavera do Leste (MT), and was analyzed using the high volume Instrument (HVI) to determine the following physical characteristics of the fiber: Trash= portion of the sample surface area that is occupied by non-lint material (%); UHM= average fiber length (mm); SFC= short fiber index (%); Elg= elongation (percentage of distension of the fibers, the initial distance to rupture); Mic= micronaire index; +b= degree of yellowing; Rd= degree of reflection (%); Unf= length uniformity (%); STR= rupture strength (gf tex^{-1}).

Data were subjected to analysis of variance by F test, and when significant to Tukey's test, both at 5% probability, with the help of software ASSISTAT 7.7 Beta (Silva, 2016).

RESULTS AND DISCUSSION

No significant difference was observed between the five harvest systems (Table 1). In any harvesting operation, it is common to incur losses, but in the case of cotton harvesting, this occurs because of the lack of efficiency of the harvester, which may fail to harvest cotton still present in the plant. Each harvest system demonstrated efficiency levels well below expectation. This fact is made evident by the high rate of total losses (Table 1), even

Figure 1. (A) John Deere picker harvesting with platform type Pro-12 VRS, (B) Harvester with brush-type picking platform with a view of the coupled field cleaner used in both experiments, (C) Harvester with finger type harvesting platform.

Table 1. Mean efficiency of collection systems (%), total losses (kg ha^{-1} and %) on the basis of cotton harvesting systems.

Harvest system	Efficiency (%)	Total losses (kg ha^{-1})	Total losses (%)
Brush stripper without field cleaner	89.43[a]	219.97[a]	11.92[a]
Brush stripper with field cleaner	88.91[a]	232.00[a]	12.57[a]
Pro-12 VRS picker	88.95[a]	230.27[a]	12.48[a]
Finger stripper without field cleaner	91.10[a]	182.93[a]	9.91[a]
Finger stripper with field cleaner	88.78[a]	234.77[a]	12.72[a]
Average	89.43	206.48	11.19
C.V. (%)	3.11	29.37	29.37

Averages followed by the same letter in the vertical were not statistically different by Tukey's test at 5% probability.

though the productivity of the area was 1845.78 kg ha^{-1}.

In the study carried out by Silva et al. (2007), an increase in crop losses was attributed to the fact that there are large numbers of bolls closed at harvest,

Table 2. Average bark, stem and total trash in the samples taken from the basket of the harvester (%).

Harvest system	Average (%)		
	Bark	Stem	Total trash
Brush stripper without field cleaner	19.82[c]	3.16[c]	22.98[c]
Brush stripper with field cleaner	5.89[b]	2.01[b]	7.95[b]
Pro-12 VRS picker	0.87[a]	0.32[a]	1.19[a]
Finger stripper without field cleaner	20.98[c]	3.32[c]	24.30[c]
Finger stripper with field cleaner	4.68[b]	2.36[bc]	7.04[b]
C.V. (%)	13.33	28.66	28.66

The averages followed by the same letter in the vertical are not statistically different from each other by Tukey's test at 5% probability.

Table 3. Average results of impurities area (Trash), uniformity (Unf), reflectance (Rd), yellowness (+b) and elongation (Elg) cultivar IMA 5672, samples collected in the basket of the harvester.

Harvest system	Area trash (%)	Unf (%)	Rd (%)	+b	Elg (%)
Brush stripper without field cleaner	1.37[ab]	82.9[a]	75.9[a]	8.9[a]	8.1[a]
Brush stripper with field cleaner	1.23[ab]	83.0[a]	75.5[a]	9.0[a]	7.8[a]
Pro-12 VRS picker	0.68[a]	83.1[a]	75.6[a]	9.2[a]	8.1[a]
Finger stripper without field cleaner	1.54[b]	83.0[a]	75.9[a]	9.0[a]	8.4[a]
Finger stripper with field cleaner	0.97[ab]	82.5[a]	76.8[a]	9.1[a]	7.8[a]
Average	1.4	82.6	75.2	9.1	8.0
C.V. (%)	36.56	1.5	1.62	5.2	4.9

Averages followed by the same letter in the vertical are not statistically different from each other by the Tukey's test at 5% probability.

causing the cotton inlet flow to the machine to be reduced, thereby decreasing the efficiency of the harvester. However, this fact does not corroborate the data from this study because even with the sum of the half-open and closed bolls, there were 95.57% more open bolls. According to Ribeiro et al. (2012) a proportion of 90 to 95% bolls open is adequate for starting the harvest.

To determine the content of impurities present in the cotton sample, taken from the basket of the harvester, the percentage of bark and stem was quantified and according to the data presented in Table 2, the Pro-12 VRS picker platform had the lowest percentage of bark (0.87%) and stem (0.32%). The absence of impurities in the extractor stripper harvester brush and finger provided an increase of 70.3 and 77.7% bark and 36.4 and 28.9% stem, respectively, compared with the brush stripper platform and finger with the presence of the field cleaner. According to Faulkner et al. (2007), the foreign matter can be reduced by using a cleanser, but the levels of these materials are still higher than those found in the cotton harvested with a picker, corroborating data from this study. The Pro-12 VRS picker platform, compared to the brush and finger crop platforms with field cleaner, decreased the presence of stems by 84.08 and 86.21%, respectively.

In general, the Pro-12 VRS picker harvesting system yielded less waste than the waste stripper harvester. Comparing the stripper harvester, the absence of field cleaner afforded an increase of 65.4 and 71.0% in the presence of garbage in the brush and finger platforms, respectively. In regards to the physical characteristics of the fiber, only the trash was influenced by the harvesting system. As expected, the percentage of total trash was higher in stripper harvesting systems than in picker harvesting systems. The other variables were not affected by the type of harvesting platform used.

There were significant differences between different harvest systems in regards to the percentage of area occupied by impurities, with a level of significance of 5% (Table 3). Cotton harvested with the Pro-12 VRS picker platform had the lowest percentage of impurities within a trash (0.68%), differing significantly only from the comb stripper platform without field cleaner, which, containing 1.54% impurities, had a greater presence of impurities than any other harvest system. However, there was a trend of stripper harvesters without the presence of field cleaner having a higher percentage of impurities.

However, in the cotton samples harvested by a brush stripper platform without field cleaner, a brush stripper

Table 4. Average results of fiber length (UHM), strength (STR), short fiber index (SFI), and micronaire (Mic) for cultivar IMA 5672, for samples collected from the basket of the harvester.

Harvest system	UHM (mm)	STR (gf tex^{-1})	SFI	Mic (μg/in)
Brush stripper without field cleaner	27.1[a]	27.3[a]	8.9[a]	3.9[a]
Brush stripper with field cleaner	27.1[a]	28.0[a]	8.6[a]	3.9[a]
Pro-12 VRS picker	26.9[a]	27.8[a]	8.7[a]	4.0[a]
Finger stripper without field cleaner	27.0[a]	28.0[a]	7.6[a]	4.1[a]
Finger stripper with field cleaner	27.2[a]	28.9[a]	8.9[a]	4.0[a]
Average	27.3	28.4	8.9	3.9
C.V. (%)	3.10	4.70	14.14	4.90

Averages followed by the same letter in the vertical are not statistically different from each other by the Tukey's test at 5% probability.

with field cleaner, and a finger stripper without field cleaner, the samples were still considered to contain a high percentage of impurities in the samples (> 1.0) (Lamas, 2004). The uniformity of fiber length (Unf) averaged 82.6% that is, falling into the category of high Uniformity Index Fiber Length (Table 3). These values exceed the standard by what the textile industry considers an ideal fiber length uniformity index, ranging between 80 and 82% (Bolsa de Mercadorias and Futuros, 2002).

Reflectance degree (Rd) indicates how much gray or light is the sample. Cotton fiber ranges from 40 to 85 Rd. In this study, the average Rd was 75.9, with one small variation. The degree of yellowness (+b) indicates yellow in the sample. Cotton fiber ranges from 4 +b to 18 +b, and had an average of 9. For the variable elongation (Elg), regardless of study treatment, it was considered very high according to the classification (Table 3).

Industrial demand for fiber length is higher than 28 mm (Freddi et al., 2014), and at an average of 27.4 mm, fiber lengths in this study were lower (Table 4). The characteristic strength (STR) is within the industry compliance standards, since the required standard is more than 28 gf tex^{-1} and the average STR found in this study was 28.4 gf tex^{-1}, that is their classification fits as medium resistance (Table 4).

The rate of short fibers (SFI) showed an average of 8.5, a low value considering the category. For marketing, values above 10% are considered unfavorable by the market. The fiber micronaire index (Mic), falls within the "fine" category for the brush stripper harvester with and without field cleaner, while the others classify as "average" (Table 4).

Conclusion

Harvesting high density cotton results in a significantly smaller amount of waste when harvested with the Pro-12 VRS picker than with the stripper harvesting system. Field cleaner of the stripper harvester with brush and comb platforms provide cotton with a smaller amount of stem and bark. The Pro-12 VRS picker harvester failed to preserve the intrinsic quality of the fiber.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Nutritive value and degradability "in situ" of dry matter elephant grass silages with addition of babassu meal

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The goal of this study was to evaluate the nutritive value and the in situ degradability of elephant grass (*Pennisetum purpureum* Schum) silages with addition of babassu meal (BaM). PVC silos with 0.25 m in diameter and 0.75 m in height were used. It was done by adopting a compression of 550 kg/m^3 in a completely randomized design with five repetitions. It was found that increasing linear effect (P< 0.05) on the average content of silage DM, estimating an increase of 0.74% for each unit added. The crude protein content of the silage without BaM was lower than the fresh grass. There was a linear effect on the levels of neutral detergent fiber, with an estimated reduction of 0.17% per unit added. Acid detergent fiber and lignin showed linear response to increasing levels of BaM. Disappearance of DM was significant to the extent that the material remained in the rumen, the best disappearance values were observed in the time of 72 h for all levels of inclusion and among the levels of inclusion of 20% showed the best values of the disappearance of DM at all incubation times. The bran of babassu oil improves the nutritive value, the degradation parameters and provides better conservation of ensiled mass.

Key words: Additive, conservation, by-product, *Pennisetum purpureum.*

INTRODUCTION

The use of silages of tropical grass has become very common in the production of ruminants, as a way to use the surplus of forage production of rainy period of the year to minimize the problem of food shortage in the dry period. Among the perennial grasses, the elephant grass is one of the most used alternatives. Its use is indicated mainly by reason of their characteristics of production of dry matter and of its nutritional value. Santos et al. (2010) stated that the silage tropical grass is an alternative for silage of traditional cultures and has as advantages the use of perennial crops and the use of the surplus produced in the season of the waters.

The tropical forage grasses not present appropriate levels of dry matter (DM), soluble carbohydrates and

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Table 1. Chemical composition of elephant grass and the babassu meal.

Grass	Chemical composition (%Dry matter)					
	DM[1]	CP[2]	NDF[3]	ADF[4]	Lignin	Hemicellulose
Elephant grass	17.9	9.5	72.20	35.02	43.08	31.94
Babassu meal	94.25	16.85	68.98	34.73	31.69	33.04

[1]Dry matter, [2]Crude protein, [3]Neutral detergent fiber, [4]Acid detergent fiber

values of power plug that provide efficient fermentation process. Grass silage with less than 21% of DM, soluble carbohydrates below the 2.2% in green and low soluble carbohydrates and power plug presents a greater possibility of secondary fermentation, which in turn are undesirable.

In this sense, Zopollatto et al. (2009), analyzing various work on silage of tropical forage grasses, cited data that the low levels of DM and soluble carbohydrates in the ideal time of harvest. These situations can be modified by the use of techniques such as mixing of products to earth grass silage (additives) or by partial withdrawal of the water of the plant by wilting.

In this context, the use of vegetables by-products regional become compelling alternatives to reduce the cost in animal feed and reduce the negative impacts of when this material is discarded into the environment. The babassu meal (BaM) is a by-product found in large scale in the state of Maranhão, in view that the same are the biggest areas of babassu palm of Brazil. Its importance increases even more by their exploitation represent a form of income of more than 400 thousand families in Maranhão, Brazil.

Even with great emphasis on the economy of the states of the Union, is a by-product that needs more studies on the exploitation of its potential, since its economic utilization is connected to the extraction and exploitation of oil of coconut of babassu rejecting 90% of the fruit, that can be leveraged with the technologies available, either as an energy source, as raw material for the steel industry, of food, animal nutrition, among others.

Of meals produced in Brazil the babassu meal is little used in animal feed, due to its very varied, bromatologic composition and to be produced on a small scale, its greater use is in the north and northeast regions that are the largest producers of fruit.

Given the importance of the use of the silages in ruminant feed this work aimed to evaluate the nutritive value and the in situ degradability of dry matter of elephant grass silages with different proportions of babassu meal.

MATERIALS AND METHODS

The experiment was conducted in the sector of foraging located in the Center of Agrarian Sciences and Environmental Federal University of Maranhão, in Chapadinha. The grass was cut manually, close to the ground, when it reached an average height of 1.6 m and 45 days of age of regrowth. It was placed in a shed covered for the withering, for a period of six hours, and later was chopped into particles of approximately 2 cm, in forage machine attached to the tractor. The babassu meal was acquired in an industry oil industry located in the city of Itapecuru-Mirim, East Maranhão, Brazil.

Experimental design and treatments

The meal of babassu was added to grass newly harvested minced in five levels (0, 5, 10, 15 and 20%) of natural material of grass, with five repetitions in a completely randomized design. For fabrication of the silages were used PVC silos with 0.25 m diameter and 0.50 m in height, endowed with drains for the collection of the effluent. In each silo was placed on average 2 kg of fresh mixture, adopting a compression of 550 kg/m^3. After filling, the silos were sealed with PVC caps and coated with adhesive plastic tape. On the seventh day, after filling held that seal of existing drains at the top of each silo. The chemical composition analyzes of elephant grass and the babassu bran is presented in Table 1.

After 125 days of silage, has opened the silos, collecting then samples for each experimental unit. For evaluation of the silages bromatologic composition, samples were collected from fresh material of silos, who were submitted to pre-drying in an oven of forced ventilation at 65°C until constant weight, then were crushed in mill Willey type with 5 mm sieve.

Measurements

The dry matter content (DM), crude protein (CP) and pH were calculated according to the recommendations of the AOAC (1990), the neutral detergent fiber (NDF) and acid (ADF) following the procedures of van Soest et al. (1991). The hemicellulose was calculated by the difference between the NDF and ADF and the cellulose content by the difference between the ADF and the lignin.

For assessment of in situ degradability of DM were used nylon bags with dimensions 12 x 8 cm and porosity 50 μm (Ørskov and McDonald, 1979), containing 4 g of the sample in agreement with relation of 42 mg/cm^2 adopted by Campos et al. (2011) and incubated in the beef rumen fistulated in times 6, 24 and 72 h (NRC, 2001) in descending order of time, thereby providing withdrawal of all bags simultaneously of ruminal environment. During the experimental period, including the adjustment period of seven days, total diet was provided for maintenance, according the NRC (2001). The diet was composed of 80% roughage and 20% concentrate and supplied in two meals (at 8 and 16 h), plus mineral mixture and the water at will.

To evaluate the parameters of dry matter degradation (DDM), we used the Brody model according to the equation of Orskov and McDonald (1979) modified by Sampaio (1995): %DegMS=A-Bexp(-C*Time), in which: A = potential degradation of the forage, without time for colonization, that is, if the Deg% at time zero was 0%; B = percentage of degraded material deposited in the rumen without time for colonization; C = degradation rate constant of the material

Table 2. Chemical composition of elephant grass silages with the addition of different levels babassu meal.

Composition	Levels of addition of babassu meal (%)					Regression equation	
	0	5	10	15	20		
CP	6.56^{b}	10.33^{ab}	11.73^{a}	13.13^{a}	9.45^{ab}	$\hat{Y}$=-0.04X²+1.03X + 6.36	R²=0.75
pH	3.58^{ab}	3.58^{ab}	3.51^{b}	3.46 ^{a}	3.66^{b}	$\hat{Y}$ = -0.00098X²+ 0.02x + 3.59	R²=0.79
DM	17.38e	23.84^{d}	28.19^{c}	38.65^{b}	42.52^{a}	$\hat{Y}$= 0.002X + 24.81	R²=0.76
NFD	75.23^{b}	73.08^{b}	72.34^{ab}	71.75^{ab}	69.84^{a}	$\hat{Y}$ =0.30X + 74.57	R²=0.73
AFD	46.54^{a}	46.81^{a}	48.70^{b}	49.50^{b}	50.90^{c}	$\hat{Y}$ =0.23X + 46.16	R²=0.89
Lig	5.73^{a}	6.25^{b}	6.43^{b}	6.86^{c}	7.10^{c}	$\hat{Y}$= 0.07X + 5.82	R²=0.92
Hem	28.69^{d}	25.53^{dc}	23.05^{bc}	20.34^{c}	17.95^{a}	$\hat{Y}$=0.53X + 28.5	R²=0.72

Means in the lines followed by the same letter do not differ by Tukey's test (P<0.05).

remaining in the rumen at any incubation time.

Effective dry matter degradability (DEMS) was calculated assuming three ruminal passage rates (2, 5 to 8%/h) through the equation described by Ørskov and McDonald (1979): DE= α+(β'*C/C+k), where: a' = % disappearance at time zero (mean); β'= A-α; C = degradation rat constant rate; k = passage rate.

Statistical analysis

Initially the data were submitted to normality test (Crame-Von Misses) and scapular (Levene) and attended the assumptions, they were submitted to variance analysis by F-test. In the case of significant difference, comparison of averages was by Tukey at 5% probability. The statistical analyzes were performed by the PROC GLM/SAS 9.0 (2002).

For evaluation of degradation, descriptive statistics was performed for the average according the PROC MEANS of SAS (2002). The parameters α, β and c and the in situ degradation curves were obtained according to the equation proposed by Ørskov exponential and McDonald (1979) and determined according to the method of Gauss-Newton through the PROC NLIN of SAS (2002).

RESULTS AND DISCUSSION

The data relating to the chemical composition and regression equations in function of increasing levels of babassu meal (BaM) in silage of elephant grass are presented in Table 2.

It was observed that the silages with the inclusion of babassu meal showed higher levels of CP when compared to silage without BaM, which had its average content of CP reduced from 9.5 to 6.5% in relation to the grass at the natural grass; however there was no influence of inclusion of BaM in additions of 5 and 20%.

For the crude protein content, quadratic effect was observed with the addition of levels of BaM, estimating maximum value of 13.5% CP for the level of 12.87% from the meal. According to Sampaio et al. (2010), the silages without the babassu meal showed levels of CP, inferior to 7%, value considered as minimum level for that where there is proper functioning ruminal. Therefore, the silage without inclusion proved to be an inefficient amount of protein to animals. The low content of CP observed in silage without meal may be attributed to the loss of soluble nitrogen compounds during the withering.

It was verified to have a quadratic effect (P<0.05) levels of meal on pH, estimating minimum value of 3.46 for the level of 15% of babassu meal. It can be observed that this characteristic remained within the optimal range (3.4 to 4.2) for silages well preserved.

Also, an increase in the content of MS with the incorporation of BaM was also notably observed. To carry out the study of the regression equation, linear effect was verified (P<0.05), with an estimated increase of 0.74% in the content of DM per unit of meal added. These increases were also observed by Ferrari et al. (2009) who observed an increase in the content of dry matter of elephant grass silage with the addition of citrus pulp, plus 10% of by-product with an increase of 7.71% in DM content in the silage.

The silages with levels of 0 and 5% meal showed lower levels of DM, which associated with higher pH values recorded, could have contributed to the occurrence of a fermentation inadequate; however, this fact was not observed, since the smell of silages well fermented and firm physical appearance, because during the opening there was a test of observation by the researchers.

Silages with 20% of BaM, despite having presented a high value of DM, had a high pH which occurred due to the high content of CP that influenced buffering of the middle and prevented the pH reduction. These data indicate that the BaM appeared to be an additive efficient in raising the content of DM of silage of elephant grass, produced with high moisture content, because with only 10% of byproduct came 28.19% of DM. Such fact can be attributed, among others, to the high content of dry matter of meal (94.25%), as well as to its high hygroscopic capacity.

Pompeu et al. (2006) worked out the addition of increasing levels of pineapple by-product of the observed linear increase in the levels of dry matter of the silages. For each 1% addition of the by-product of pineapple, there were increases of 0.71% points in DM levels.

Although the moisture content has been above 70% on silage witness and inclusions of 5, 10 and 15% of BaM,

Table 3. Average values of disappearance of dry matter (%) according to the level of addition of babassu meal in elephant grass silage and the incubation time.

Level of addition of babassu meal (%)	Incubation time (hours)			CV (%)
	6:00	24:00	72:00	
0	39.0^{Cc}	46.3^{Bc}	53.0^{Ab}	4.56
5	42.4^{Cbc}	47.6^{Bbc}	54.6^{Ab}	4.21
10	47.2^{Bb}	49.8^{Bbc}	56.6^{Aab}	4.65
15	47.2^{Bb}	51.4^{ABab}	54.8^{Ab}	4.61
20	52.0^{Ba}	55.6^{ABa}	60.2^{Aa}	4.67

Means followed by letters equal uppercase (row) sensitive (columns) do not differ by the Tukey's test (P<0.05).

there were apparently unobserved losses arising from possible undesirable fermentations by bacteria heterolactic or even by rot common in wet silages. The silages showed pleasant aroma and in all of them was observed the characteristic smell of homolactic silages.

A decreasing linear effect (P<0.05) levels of babassu meal was observed on the levels of NDF silages. This reduction in the levels of NDF silages may be related to the use of part of hemicellulose as substrate for the fermentation; moreover, these declining trends are assigned to lower content of NDF from the meal (64.73%) in relation to the elephant grass (75.02%).

To compare the contents of NDF of grass at the moment of the silage with the silage without meal, there are values of 75.02 and 75.23%, respectively. This difference was caused by losses of soluble components of dry matter, MCdonald (1991), increasing the concentration of components of the fibrous fraction.

There was also an increase in ADF as the meal was included, being that for contents of ADF, no difference was observed between the silages without inclusion and 5%. Also, we observed increases in order of 0.21% units to each addition of 1% of BaM. These data are reports of Vieira et al. (2007), who observed a linear reduction in the levels of ADF with the addition of BaM to silages.

Elevations of ADF observed may compromise the nutritional value of the silages already mentioned by second Van Soest (1994) as there is a negative correlation between high ADF producers with the digestibility of dry matter, since an increase in the cell wall components less digestible (cellulose and lignin) by ruminal bacteria was observed. This reduction was due basically to the decrease in the NDF content of the silages, since there was no effect only at level 20% of BaM about the content of ADF silages. As the reduction in the fat content of hemicellulose was proportional to the reduction in the content of NDF, there was no loss of hemicellulose by fermentation.

The lignin content of the silages showed linear response ascending (P<0.05) levels of babassu meal, which varied from 5.73 to 7.10% in the levels of 0 and 20% of BaM, respectively. The content of hemicellulose silages showed a decreasing linear response (P<0.05) levels of babassu meal. The results obtained for the disappearance of dry matter are presented in Table 3. The disappearance of DM was significant to the proportion with which the material remained in the rumen, and the best values were observed at 72 h for all levels of inclusion.

Among the inclusion levels, 20% showed the best values of disappearance in all incubation times (6, 24 and 72 h), followed by the inclusion of 15% of babassu meal, this being less than 9.61% (6 h), 7.55% (24 h), 8.99% (72 h), however at the time of 72 h 15% of inclusion was only 5.98% less as compared to 20% of inclusion.

The lesser disappearance of dry matter (DDM) was noted in the treatment without the inclusion of babassu meal. At 6 and 24 h, already in the time of 72 h there was no significant difference (P>0.05) between treatment 5 and 15%. Despite the DDM is lower in treatment 0%, the curve of degradation was more accentuated because the degradation was slower, that is, for the time 6 to 72 h the disappearance of DM was 26.41%, that is, 48.43% higher than the treatment of 20% of inclusion of babassu, since from the time 6 to 72 h, the disappearance of DM was 13.62%.

Rêgo et al. (2010), working with the inclusion of the penduncle of cashew dehydrated in silage of elephant grass, an ascending linear behavior was observed for the same time of incubation studied in the present work; 0.64% points for each 1% of inclusion of the by-product of the cashew.

For all treatments, it was noted that with the increase in the level of inclusion of BaM in elephant grass silage the disappearance of DM from beginning to end tends to be smaller, since this material becomes much more usable quickly in time to lower incubation in relation to low levels of inclusion, being the main characteristic of the nutritive value of the silage which tends to be influenced by the increase of BaM. In Table 4 are presented the values for the parameters of ruminal degradation.

With the inclusion of 5 and 10% of babassu bran obtained values of potential degradability (A) higher than the other treatments with 69 and 68% respectively, however, presented degradation rates (C) low (< 0.025), what influenced in effective degradability (ED) in three

Table 4. Ruminal degradation parameters *in situ* dry matter of silage of elephant grass with addition of levels babassu meal.

Level of addition of babassu meal (%)	Ruminal degradation parameters						
	α	β	$C.10^2$	R^2	ED2%	ED5%	ED8%
0	53.0	34	4.0	98.5	47.02	43.04	41.05
5	69.0	39.0	1.0	97.8	49.72	44.90	43.29
10	68.0	33.0	1.0	97.7	52.86	49.08	47.82
15	61.0	25.0	2.0	98.3	53.43	50.19	48.89
20	61.0	20.0	4.0	97.4	57.41	55.02	53.83

α = Soluble fraction or readily degradable (%); β = Insoluble fraction or slowly degradable (%); c = Rate of degradation of β ($\%h^{-1}$); R^2= Coefficient of determination; ED= Effective digestibility for passage rates of 2, 5 and $8\%h^{-1}$.

rates of passages of the two treatments: 2% (49.72 on 52.86%), 5% (44.90 on 49.08%) and 8% (47.82 on 43.29%), simultaneously. According to Borges (1997), the fodder feature more digestible high values of 'A', but also need the high values of 'c', for which they achieve the maximum potential degradation in less time.

The parameters 'a' and 'c' are the main in the qualification of a forage harvester. A high value 'A' indicates a very degradable material, while greater value of 'c' means less time for the disappearance of potentially degradable fraction, being that fodder of high quality must submit degradation rates higher than $2\%h^{-1}$.

Among the inclusion levels, 0% presented low degradation parameters in relation to the other, despite presenting a desirable rate of degradation (4.0%) that influenced the effective digestibility values slightly below the level of 5%; in that way, the BaM increment helps in the potential degradability. However, it affects the rate of degradation, thus affecting the digestibility effectively, thereby identifying, explicitly, two of the main elements of qualification of forage harvesters, which are: the rate of degradation (C) and the potential degradability (A) (Sampaio et al., 1995).

With the inclusion of 20% of babassu meal, despite a potential degradability 11.51% less than the inclusion of 10% of babassu meal, this presented values of effective high digestibility in different rates of passages with 2% (57.41%), 5% (55.02%) and 8% (53.83%) in relation to the other, due to the rate of degradation being acceptable (4.0%).

It is observed that for all levels of inclusion of babassu meal, the values of these tend to diminish with increase in the rate of passage, which occurs because the remains of the material aims to be lower, thus complicating the accession of microorganisms to the food. In this way, there exists a lower passage rate of the largest.

Conclusions

The addition of BaM to silage of elephant grass promotes increments on DM and CP, reduces the levels of NDF, hemicellulose and increases ADF and lignin. With the inclusion of up to 20% of babassu meal, values of dry matter degradability and the parameters of acceptable ruminal were obtained. The inclusion of babassu meal did not affect the fermentation, because the silages showed pleasant aroma.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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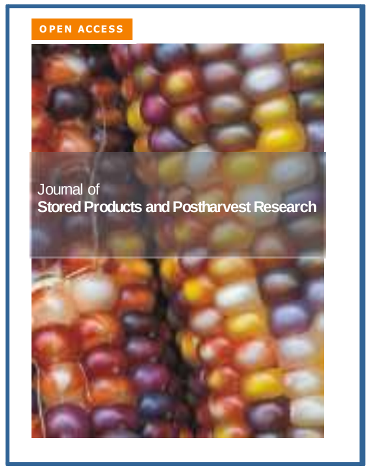

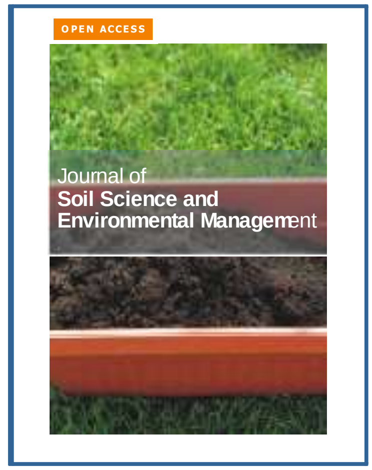

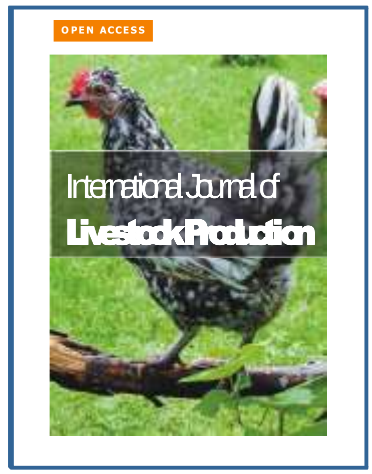

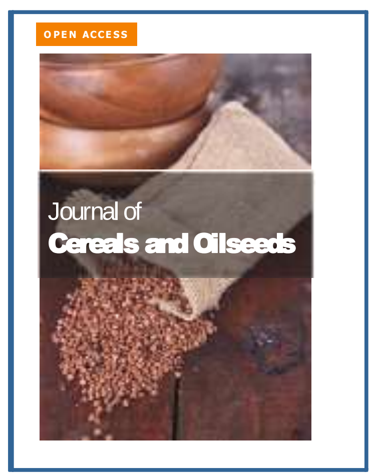

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