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Table of Content

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Full Length Research Paper

Effects of seeds ingestion by Lagune cattle and other pre-planting treatments on the germinability of *Centrosema pubescens* Benth seeds in Soudanian region of Benin (West Africa)

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In Benin under extensive management of grassland, there is little information about legumes regeneration and management in pastures. This trial evaluated the germination of *Centrosema pubescens* seeds after passage through the digestive tract of three young bulls and three heifers of *Lagune* breed cattle. Following seeds ingestion by cattle, total faeces were collected at 24-h intervals for 6 days after which time the faeces were sieved and the surviving intact seeds were then collected, counted and germination tests undertaken. Moreover, the effect of soaking seeds with hot water and mechanical scarification on breaking of dormancy in seeds of *C. pubescens* were studied through seven treatments: control (1); scarification using sandpaper (2); and seeds were immersed in hot water (80°C) for 2 (3), 4 (4), 6 (5), 8 (6), and 10 min (7). The total number of seeds recovered represented 7.65% of the number fed. The number of seeds recovered after 72 h represented more than 91.00% of total seeds recovery. Overall germination percentage of seeds recovered (45.09%) was greater than that of untreated seeds (31.00%). Seedling emergence was significantly higher when dung was broken-down than when left intact. Generally, it was observed that mechanical scarification was the method that had the highest percentage of germination (96.00%), followed by seeds immersed in hot water at 80°C for 2 to 4 min and seeds ingested by cattle. Therefore, endozoochory and other pre-planting seeds treatments can potentially favour seed germination of *C. pubescens* and contribute to the improvement of degraded grassland.

Key words: *Centrosema pubescens*, sandpapering, endozoochorous, hot water, germination, Benin.

INTRODUCTION

In tropical regions, grassland is secondary habitats formed due to human activities resulting in degradation of deciduous forests (Mandar, 2016; Houndjo et al., 2018a). Spread throughout south and north of Benin, natural savannahs and fallows are important from economic and ecological points of view and are the prime source of fodder for the large population of livestock (Sinsin, 1993; Houinato, 2001; Adjolohoun, 2008; Koura, 2015; Lesse, 2016). In these grasslands, legumes species are scarce and overexploited thus leading to their low abundance whereas native grasses and weeds are largely dominant especially in the dry season (Michiels et al., 2000; Lesse, 2016). Uncontrolled use of forage legumes species leads to degradation and reduction of their habitat and population in grassland (Sinsin, 1993; Mandar, 2016). Several studies have been conducted on legume forage species adaptation to the environmental conditions in Benin considering their yield and nutritive value (Michiels et al., 2000; Adjolohoun, 2008; Babatoundé et al., 2010; Musco et al., 2016). However, under extensive management of these pastures, there is little information about legumes regeneration and management in pastures.

Centrosema pubescens (Centro) belongs to the Fabaceae family and is a perennial, training-climbing herb with strong tendency to root at nodes of trailing stems. It is native to Central America and can be grown in many tropical regions. Centro is widely used as forage and source of calcium and phosphorus to livestock (Cook et al. 2005). Centro has been identified by several authors as potential forage legume for the tropical regions (Cook et al., 2005; Adjolohoun, 2008; Houndjo et al., 2018a). Green matter yield of *C. pubescens* varies from 13.5 to 40.0 tons ha^{-1} year^{-1} (Ajayi et al., 2008; Houndjo et al., 2018b). *C. pubescens* forage is very rich in protein (19.6%) and can be used as green manure crop in rubber, coconut and oil palm plantation and its forage can be grown for stall feeding, grazing or preserved as hay or silage for use during the dry season when there is a scarcity of grazeable materials (Ajayi et al., 2008; Houndjo et al., 2018b). It can be established by oversowing in natural or artificial pastures by enrichment planting or direct seeding (Adjolohoun, 2008). However, without seeds treatment, establishment of Centro is difficult mainly due to high proportion of hard seeds (Win et al., 1975; Muhammad, 2015; Houndjo et al., 2018b). Seed is the basic agricultural input and its quality is extremely important.

Some leguminous species with hard seeds are known to survive digestion and be dispersed by ruminants including cattle and in some cases have gone on to become established and develop into being environmental weeds (Berner et al., 1995; Paynter et al., 2003). Temporal patterns in the defecation of seeds after ingestion indicate that germination increased as the length of retention in the digestive tract increased (Jolaosho et al., 2006). Mastication and/or action of acid and enzymes present in the digestive tract of cattle could separate the seed from the shell, soften seed and/or scarify the seed coat (Traveset and Verdu, 2002).

The retention time of seeds in the digestive tract varies, depending on the type of animal (Gökbulak, 2003). Some authors suggest that the larger body size and longer intestinal tract in cattle was responsible for the lower recovery rates of *Albizia saman* seeds (Jolaosho et al., 2006). Other factors such as seed characteristics, diet quality, health, age, sex, and stress level may also influence seed retention time (Raymundo et al., 2018). Lagune cattle is the main cattle breed in south of Benin. It is of the smallest cattle breeds of Benin (Gbangboche et al., 2011; Houndjo et al. 2018a) and little is known about the recovery and germination of seeds after ingestion by Lagune cattle in Coastal region of West Africa.

Cattle ingestion and later excretion of seeds (endozoochory) of *C. pubescens* as a method of seeds dispersal would have the potential to act as a low cost alternative for transport large numbers of seeds and deposit them in a germinable form into an environment suitable for establishment (Doucette et al., 2001). Dung depositions generate gaps and provide nutrients and organic matter that facilitate seedling emergence and growth (Osvaldo et al., 2010). However, other studies indicate that seed inclusion in dung can suppress seedling emergence (Uytvanck et al., 2010; Milotić and Hoffman, 2016).

As it is desirable to contribute to the rehabilitation of degraded grassland in Benin, endozoochory, mechanical scarification, acid treatment, or hot water treatment, may be of value. Sulfuric acid was reported as having the highest positive effect in breaking seed dormancy (Muhammad, 2015). However, sand papering and hot water treatments could be considered for substitution because sulfuric acid application by most farmers is not easy (Muhammad, 2015).

This study was conducted to determine:

(1) the survival and germination of *C. pubescens* seeds fed to penned Lagune cattle;
(2) the influence of sex and seed retention time in the digestive tract on seeds germination;
(3) the effect of being contained in dung for seedling emergence;
(4) the effect of treatment with hot water and mechanical scarification on the germination of untreated seeds in order to compare different methods of breaking dormancy of *C. pubescens* seeds.

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MATERIALS AND METHODS

Plant

Seeds of *C. pubescens* were selected to examine the rate of recovery and the subsequent germination after passing through the digestive tract of bovines. Seeds were obtained from the experimental study on the influence of cattle manure rates in combination with plant row spacing conducted by Houndjo et al. (2018b). Seeds were harvested in November 2016 and stored in plastic jars in ambient temperature to prevent seed quality deterioration (Razanamandranto et al., 2004). Morphological characteristics of seeds are shown in Table 1. *C. pubescens* seed mean number per pod and thousand-seed weights were 18.09 and 34.62 g, respectively. There were 27809 to 33761 seeds/kg, with an average of 28885 seeds/kg (Table 1). The investigation involved three complementary activities which included (a) retrieval of seeds from dung and their germination testing, (b) seedling emergence directly from dung, and (c) seed treatment with mechanical scarification or hot water.

Seeds retrieved and germination after the digestive process

The first part of the investigation examined the survival and germination of *C. pubescens* seeds fed to penned cattle.

Seeds ingestion procedure

six cattle (*Bos taurus)* namely 3 young bulls (90.20 ± 5.30 kg) and 3 heifers (70.50 ± 3.50 kg) of Lagune cattle breed were used to examine seeds recovery and germinability after passage through the digestive tract of cattle. Mean age of cattle were 18 ± 2 months. Lagune cattle were used as it is the main cattle breed in the region (Gbangboche et al*.,* 2011; Assogba et al., 2016). The feeding experiment was conducted during 2 months (from December 2017 to January 2018) in the farm of the Faculty of Agricultural Sciences of the University of Abomey-Calavi at Sékou located between 6° 21' - 6° 42' N and 2° 13' - 2° 25' E. The area has a sub-equatorial climate with two rainy seasons alternated with two dry seasons of unequal duration (Houndjo et al., 2018a).

Before seeds ingestion began, each cattle was housed in an individual pen for an adaptation period of two weeks, they were treated to control ectoparasites and endoparasites. This procedure was done to avoid any stress behavior due to the captive environment that could influence or perturb any feeding or digestion aptitude. During this period, the animals were regularly fed *ad libitum* with *Panicum maximum-Stylosanthes hamata* forage mixture (70:30, dry matter basis) (Doucette et al*.,* 2001). Animals received also fresh water and mineral blocks salt *ad libitum*. This was done to be sure that they did not accidentally ingest other seeds than the ones from the study species. The same food was supplied in the same way until the end of the experiment.

The ingestion procedure required that seeds were directly placed into the oesophagus of the animals. To keep tract of recovered seeds from the dung and to obtain a sufficient number of replicates, seeds of *C. pubescens* were supplied to 3 young bulls and 3 heifers, kept individually in pens. Quantities of seeds containing exactly 1000 seeds were put into a 65 cl glass bottle mixed with 50 cl of water. The neck of the bottle was introduced into the mouth and shaken to be sure that all seeds were ingested by each of the animals individually in pens. No seed was observed to be spat out during the experiment period. Seeds therefore suffered no mechanical damage during ingestion but could be ruminated later (Gardener et al., 1993a).

Table 1. Pod length and weight of seeds of *Centrosema pubescens* used in the study.

Parameter	Average	Range
Pod length (cm)	15.66	6.00 - 17.30
Number of seeds/pod	18.09	8 - 23
1000 seeds weight (g)	34.62	29.62 - 35.96
Number of seeds/kg	28885	27809 - 33761

Source: Houndjo et al. (2018b).

Dung collection and extraction of seeds

The dung of each animal was separately and daily collected in plastic trays at 24-h intervals, during 6 days after seed ingestion. The collection ended after 6 days because previous trial showed that no more seeds were found in the dung after 5 days (Razanamandranto et al., 2004; Gardener et al., 1993a). All dung was collected from each pen at the end of each 24-h period, that is, at 24, 48, 72, 96, 120 and 144 h after consuming the seeds. Total fresh dung in each tray was weighed and mixed individually for 15 min prior to sampling. Three, 200 g sub-samples were sampled for each cattle by collection time. One sub-sample was retained for dry matter (DM) determination, one for germination assays, and another for seed recovery estimates. *C. pubescens* seed recovery was conducted using a method similar to Jones and Bunch (1977). According to this, the 200 g faecal sub-sample was placed in a 2-L container. Then 1 L of water was added and gently mixed to create a slurry, which was flowed down through a series of 2 stacked sieves with decreasing apertures of 1 and 0.42 mm. Sieves were gently washed with water, then seeds and the larger particles of digesta were sifted from the fine particles. The seeds and the larger particles that remained from each screen were transferred to trays lined with paper towel and dried for approximately 24 h at 28°C in a forced air oven. Each sample of dried faeces was carefully inspected, and all identified seeds were retrieved and separated according to seed type (Doucette et al., 2001). Whole undigested seeds were separated from broken seeds, counted and retained for germination testing in the laboratory (Jolaosho et al., 2006). Seed recovery for each day was calculated on the basis of the overall faecal collection for that time and seed densities from the 200 g sub-sample, as follows:

$$TNRS = \left[\frac{NRS_{(200g)}}{200} \right] \times TFO$$

where *TNSR* = Total Number of Recovered Seed of the period, $NRS_{(200g)}$ = Number of Recovered Seeds from the 200 g sub-sample of the period, and *TFO* = total faecal output of the period.

The percentages of seeds recovered were determined as follows:

$$SR(\%) = \left(\frac{NRS}{1000} \right) \times 100$$

where *SR* (%) is seed recovered in percentage, *NRS* refer to the cumulative number of recovered seeds up to 96 h after ingestion and 1000 the number of seed ingested.

Germination testing

All intact seeds recovered in the cattle trial were submitted to germination. Three replicates of a split-plot experiment were used with retention time (24, 48, 72 and 96 h) as the main plots in a randomized complete block design (RCBD), sex (male and female) as the subplots combined to give a total of eight treatments with 24 plots. Prior to germination test, seeds of all replicates were disinfected and rinsed with sterile distilled water (Grande et al*.,* 2013). Soil of the experimental site was collected and sieved to remove stones, leaves, stems and other materials. Due to small number of seeds recovered, for each treatment and for each replicate, all intact seeds recovered per day and per cattle were placed on the surface of 150 mm diameter pots filled with sieved, sterilized sand and kept moist. The pots were kept in a shade house at ambient temperature (approximately 29/20°C). The number of seed that germinated was recorded daily for 20 days. Seeds were considered germinated when the radicle had emerged through the integument (ISTA, 1996). Germinated seeds were removed after each count. At the end of the test, seeds that had not germinated were categorized into hard and dead components by touching and piercing with a needle. Hard seeds could not be pierced with the needle (Hassen et al., 2004).

Three parameters were calculated:

(1) Germination percentage (*GP*) was calculated with the formula:

$$GP(\%) = \frac{\sum_{i=1}^{20} ni}{20} \text{x}\, 100$$

where n_i = number of seeds germinated and removed on day *di* and *i* = 1… *i* = 20 the duration in days of the test. 20 = number of seeds of each repetition placed on each pot at the beginning of germination test.

(2) Germination speed (GS) was calculated following the formula given by Czabator (1962) as follows:

$$GS(seed/day) = \sum_{20}^{1} \frac{ni}{di}$$

where n_i = number of seeds germinated and removed on day *di*.

(3) Mean germination time (MGT), was calculated as formula given by Ellis and Roberts (1981) as follow:

$$MGT(day) = \left[\frac{\sum_{i=1}^{20} ni\,di}{\sum_{i=1}^{20} ni} \right]$$

where n_i = number of seeds germinated and removed on day *di*, and 20 the duration in days of the test.

Seedling emergence from dung

The second part of the investigation observed the effect of being contained in dung for seedling emergence. Three replicates of a split-plot experiment were used with retention time (24, 48, 72 and 96 h) as the main plots in a randomized complete block design (RCBD), sex (male and female) as the subplot, dung form (broken down and intact) as the sub subplots combined to give a total of 16 treatments with 48 plots. A sampling of 100 g fresh dung from each day and from each animal (generated in feeding activity) were placed in soil on a tray (12 cm width × 20 cm long × 8 cm depth) and was placed outdoors and left for 2 months. The faeces were either broken down the soil surface (simulating crumbling of dung by rainfall or by animals) or left intact on the soil surface (Mancilla-Leytón et al*.,* 2012). The soil was kept moist. After two months, the number of plants emerging from each faeces was counted (Ghassali et al., 1998).

Other pre-planting seeds treatment of *C. pubescens*

For the third part of the investigation the effect of soaking seeds with hot water and mechanical scarification were studied in a split-plot design replicated 5 times. There were seven treatments: control (1); Sand paper: mechanical scarification using sand paper (2); and seeds were immersed in hot water (80°C) for 2 (3), 4 (4), 6 (5), 8 (6), and 10 min (7). For each treatment and for each replicate, 20 seeds were placed on the surface of 150 mm diameter pots filled with sieved, sterilized sand and kept moist and the germination test were conducted using the standard procedure mentioned above (ISTA, 1996).

Statistical analysis

The General Linear Model procedure of SAS (SAS Institute Inc. 1989) was used for analysis of variance of the germination parameters in the first and third part of the investigation. For the first part eight treatments [4 retention times (24, 48, 72 and 96 h) × 2 sex (male and female)] were considered. In both activities, the total percentages of germinated, hard and rotten seeds were subjected, after arcsine transformation, to analysis of variance using Proc GLM of SAS (1989). When Fisher's F values were significant at P < 0.05, the analysis was continued by comparing the means using Tukey's test at the threshold of P < 0.05. Arcsine-transformed means were back transformed for presentation. In the second part of the investigation the number of *C. pubescens* seedlings emerged from intact and crumbled faeces was compared using t-test.

RESULTS

Faeces recovery and number of seeds recovered from cattle

The data of number of seeds fed to the cattle that were recovered intact from the faeces was presented in Figure 1. A mean of 1.13 kg DM of faeces per animal male and 1.09 per animal female was produced over the 120 h period. The total number of seeds recovered from young bull (71.37 seeds) and heifer (81.63 seeds) accounting for 7.14 and 8.16% of the seeds ingested by the cattle, respectively were not significantly different (p>0.05) (Figure 1). The average number of seeds recovered from the cattle at the end of 96 h represented 7.65% of the number fed. There was a definite pattern of excretion of seeds by the cattle, with a distinct peak during the 48 to 72 h after ingestion (Figure 1). The number of seeds recovered after 72 h represented more than 91.00% of total seeds recovery. Seeds recovered during the 48 to 72 h after ingestion from young bull (63.91 seeds) and heifer (70.34 seeds) was significantly (p<0.05) higher than the numbers recovered at other times for all cattle

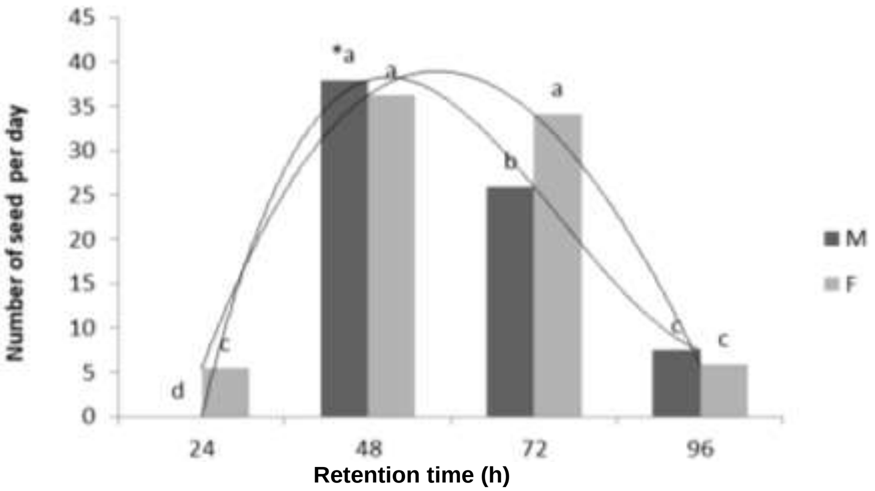

Figure 1. Number of seeds per head recovered from cattle faeces. *Different letters indicate significant differences among number of seeds per head recovered from cattle faeces (Tukey test; p<0.05).

(Figure 1). While the numbers of seeds recovered from the heifer at 24 h (5.43 seeds), and 96 h (5.86 seeds) was similar and significantly lower than those recorded at 48 or 72 h (Figure 1).

Seed germination after ingestion by cattle

Mean effects of ingestion by cattle on germination are shown in Table 2 and Figure 2. All three germination indices (percentage of germinated seeds, mean germination time and germination speed) were significantly influenced by the retention time in digestive tract of cattle (p<0.05). Overall, germination percentage of seeds recovered from faeces (45.09%) was significantly higher (p<0.05) than that of untreated seeds (31.00%) (Figure 2). Percentage of germinated seeds of *C. pubescens* did not increase, as the retention time increased from 48 h (47.83%) to 72 h (43.50%) (Table 2). The analyses also revealed a significant retention time × animal sex interaction for percentage of germinated seeds of seeds recovered from faeces 48 h after ingestion. Germination speed significantly decreased (p<0.05) as the retention time increased from 48 h (4.24 seeds/day) to 72 h (0.95 seed/day) (Table 2).

Hard and germinable levels of seeds were also determined before and after passage through the cattle (Tables 3 and 4). Before passage through the cattle tract, the amount of hard seeds (53.00%) was significantly (p<0.05) higher than the amount of germinable seeds (31.00%) (Table 3). After seed passage through cattle tract, the proportion of hard seed 38.99% (61.14 divided by 156.80; Table 4) was significantly lower than germinable seed 46.73% (73.27 divided by 156.80; Table 4). However, as increasing times of excreted seeds, the fraction of germinable seeds in the recovered fraction was lower and the fraction of hard seeds higher (Table 4). Germinable seeds (164.00 seeds), hard seeds (117.50 seeds) and rotten seeds (60.28 seeds) recovered at 48 h after ingestion was significantly (p<0.05) higher than those recovered at other times for all cattle (Table 4). Germinable seeds (8.57 seeds), hard seeds (11.17 seeds) and rotten seeds (1.77 seeds) recovered at 96 h after ingestion was significantly (p<0.05) lower than those recovered at other times for all cattle (Table 4). The total number of germinable seeds from young bull (253.00 seeds) and heifer (259.88 seeds) at the end of 96 h were not significantly different (p>0.05) (Table 4).

Establishment in trays of *C. pubescens* seedlings from faeces

Number of *C. pubescens* seedlings emerged from intact and crumbled faeces is shown in Figure 3. The number of seedlings emerging out of crumbled faeces (7 plants per cattle) was significantly higher (p<0.05) compared to seedling emergence out of intact faeces (2 plants per cattle) (Figure 3). Additionally, the day of faeces collection had influence on seedling number. Number of seedling emergence from crumbled faeces at 48 h (4.35 plants per cattle) was significantly higher (p<0.05) than the numbers of seedlings recovered at other times for all cattle (Figure 3). There was no seedling emergence in intact faeces at 24 h after ingestion.

Effects of pre-planting seed treatment on germination parameters

The results of the germination test for other methods (soaking in hot water or sand paper) of breaking

Table 2. Characteristics of seeds germination after passage through digestive tract of cattle and faeces dry matter per cattle per day.

Retention time (h)	Sex	Percentage of germinated seeds (%)	Mean germination time (day)	Germination speed (seed/day)	Faeces dry matter (g)
24	Young bull	0^{Bd1}	0^{Bd}	0^{Be}	214.39^{Ad}
	Heifer	50.00^{Aa}	7.00^{Aa}	1.08^{Ac}	55.96^{Be}
	Mean	25.00	3.50	0.54	135.17
48	Young bull	55.00^{Aa}	6.64^{Aa}	4.74^{Aa}	108.66^{Ac}
	Heifer	40.67^{Bb}	7.22^{Aa}	3.74^{Bb}	108.36^{Ac}
	Mean	47.83	6.93	4.24	108.51
72	Young bull	44.33^{Ab}	5.75^{Ab}	0.87^{Acd}	221.35^{Ad}
	Heifer	42.67^{Ab}	6.16^{Ab}	1.03^{Ac}	216.73^{Ad}
	Mean	43.50	5.95	0.95	219.04
96	Young bull	33.00^{Bc}	7.00^{Aa}	0.08^{Be}	284.87^{Bb}
	Heifer	50.00^{Aa}	2.00B^{c}	0.50^{Ad}	348.00^{Aa}
	Mean	41.50	4.50	0.29	316.43
Overall mean		45.09	5.96	1.72	194.79

[1]For the same column, means followed by the same lower letter are not significantly different ($p<0.05$). Within the same column and for the same retention time, means followed by the same upper letter are not significantly different ($p<0.05$).

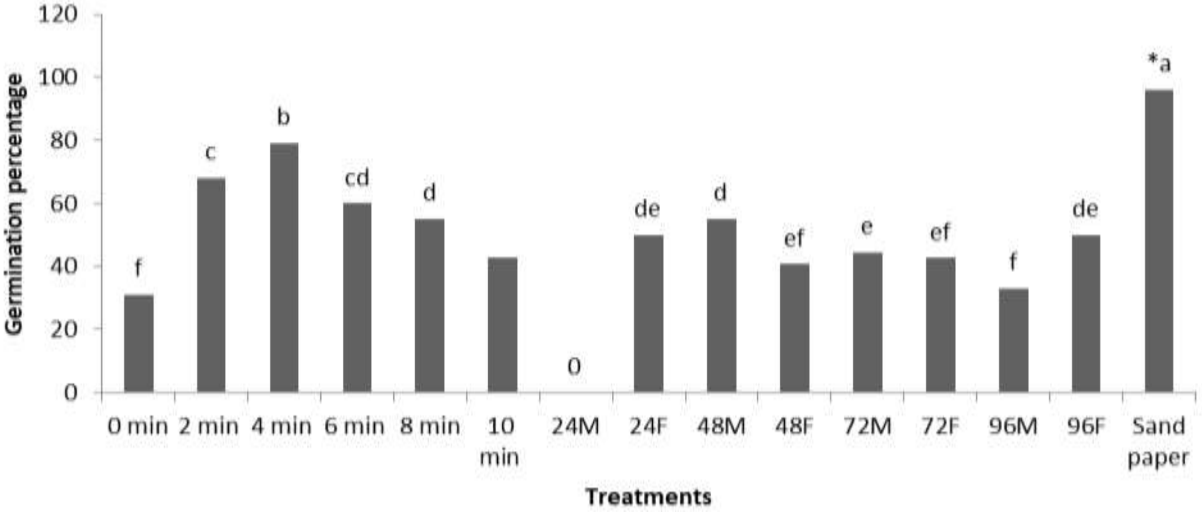

Figure 2. Germination percentage of treated *C. pubescens* seeds. (Sand paper: mechanical scarification using sand paper; and seeds were immersed in hot water (80°C) for 2, 4, 6, 8, and 10 min. Also seeds recovered in the cattle trial with retention time (24, 48, 72 and 96 h) and sex (male (M) and female (F)) were combined to give a total of eight treatments (24 M, 24 F, 48 M, 48 F, 72 F, 72 M, 96 M, 96 F). *Different letters indicate significant differences among Germination percentage of treated *C. pubescens* seeds (Tukey test; $p<0.05$). 0: In these time periods no seeds were recovered.

dormancy of *C. pubescens* seeds are shown in Figure 2 and Table 3. There was significant difference ($p<0.05$) in parameters measured between treatments. In the present study, as increasing soaking time, percentage of germination and germination speed values were increased and peaked at 4 min soaking. However, with the longer time of exposure to hot water, the values of the two germination indices were decreased (Figure 2 and Table 3). Mechanical scarification with sand paper significantly increased ($p<0.05$) seeds germination compared to control, hot water scarification or passage through cattle tracts (Figure 2). Mechanical scarification was the method which revealed to be more efficient to remove seed dormancy as it had the highest percentage

Table 2. Characteristics of seeds germination after passage through digestive tract of cattle and faeces dry matter per cattle per day.

Retention time (h)	Sex	Percentage of germinated seeds (%)	Mean germination time (day)	Germination speed (seed/day)	Faeces dry matter (g)
	Young bull	0^{Bd1}	0^{Bd}	0^{Be}	214.39^{Ad}
24	Heifer	50.00^{Aa}	7.00^{Aa}	1.08^{Ac}	55.96^{Be}
	Mean	25.00	3.50	0.54	135.17
	Young bull	55.00^{Aa}	6.64^{Aa}	4.74^{Aa}	108.66^{Ac}
48	Heifer	40.67^{Bb}	7.22^{Aa}	3.74^{Bb}	108.36^{Ac}
	Mean	47.83	6.93	4.24	108.51
	Young bull	44.33^{Ab}	5.75^{Ab}	0.87^{Acd}	221.35^{Ad}
72	Heifer	42.67^{Ab}	6.16^{Ab}	1.03^{Ac}	216.73^{Ad}
	Mean	43.50	5.95	0.95	219.04
	Young bull	33.00^{Bc}	7.00^{Aa}	0.08^{Be}	284.87^{Bb}
96	Heifer	50.00^{Aa}	$2.00B^{c}$	0.50^{Ad}	348.00^{Aa}
	Mean	41.50	4.50	0.29	316.43
Overall mean		45.09	5.96	1.72	194.79

[1] For the same column, means followed by the same lower letter are not significantly different ($p<0.05$). Within the same column and for the same retention time, means followed by the same upper letter are not significantly different ($p<0.05$).

Table 3. Characteristics of seeds germination for the various other pre-planting seeds treatments.

Treatment	Germinable seeds (%)	Hard seeds (%)	Rotten seeds (%)	Mean germination time (day)	Germination speed (seed/day)
0 min	31.00^{f1}	53.00^{a}	16.00^{c}	3.59^{a}	2.44^{c}
Sand paper	96.00^{a}	0.00^{d}	4.00^{d}	1.75^{b}	13.26^{a}
2 min	68.00^{c}	17.00^{b}	15.00^{c}	4.87^{a}	5.06^{b}
4 min	79.00^{b}	9.00^{c}	12.00^{cd}	4.11^{a}	6.53^{b}
6 min	60.00^{cd}	10.00^{c}	30.00^{b}	3.82^{a}	5.31^{b}
8 min	55.00^{d}	12.00^{bc}	33.00^{b}	4.67^{a}	2.84^{c}
10 min	43.00^{e}	8.00^{c2}	49.00^{a}	4.64^{a}	2.01^{c}
Overall mean	61.71	15.57	22.71	3.92	5.35

[1] For the same column, means followed by the same lower letter are not significantly different ($p<0.05$).

of germination (96.00%) and germination speed (13.26 seeds/day). In contrast, it had the lowest mean germination time values (1.75 day). This method was followed by seeds immersed in hot water for 2 to 4 min and seeds ingested by cattle (Table 2 and Table 3). The lowest percentage of germination (31.00%), were recorded in control (T0), the highest mean germination time (5.96 days) and the lowest germination speed (1.72 seed/day) values were recorded in seeds ingested by cattle (Table 2 and Table 3).

DISCUSSION

The investigation involving three complementary activities were conducted to facilitate the improvement of degraded grasslands in Benin. The first part of the investigation examined the survival and germination of *C. pubescens* seeds fed to penned cattle.

Since the seeds were supplied directly into the esophagus of cattle, they were not exposed to chewing during ingestion. Seeds therefore suffered no mechanical damage during ingestion but could be ruminated later (Gardener et al., 1993a). Seeds are damaged by masticating (Ozer, 1979). Also, the chewing time during rumination is much greater compared to chewing time during ingestion (Minson, 1990; Babatoundé, 2005). So, the percentage of seeds recovered from faeces is likely to be greater than when seeds are fed to animals (Gardener et al., 1993a). However, the influence should be small because much of the damage to ingested seeds occurs in the rumen and some in the abomasum (Simao et al., 1987). The average number of seeds recovered from the cattle at the end of 96 h represented 7.65% of

Table 4. Distribution of germinating, hard and rotten seeds after excreted seeds in 1000g DM faeces per day.

Retention time (h)	Sex	Excreted seeds in 1000 g DM	Germinable seeds	Hard seeds	Rotten seeds
	Young bull	0.00^{Be}	0.00^{Bd}	0.00^{Bh}	0.00^{Bb}
24	Heifer	97.00^{Ac1}	48.52^{Ac}	27.33^{Ae}	21.16^{Ab}
	Mean	48.50	24.26	13.66	10.58
	Young bull	349.20^{Aa}	192.20^{Aa}	94.33^{Bb}	62.65^{Aa}
48	Heifer	334.30^{Aa}	135.75^{Bb}	140.67^{Aa}	57.90^{Aa}
	Mean	341.80	164.00	117.50	60.28
	Young bull	117.20^{Bc}	52.08^{Ac}	59.67^{Bd}	5.49^{Ab}
72	Heifer	157.30^{Ab}	67.18^{Ac}	83.68^{Ac}	6.43^{Ab}
	Mean	137.30	59.63	71.67	5.95
	Young bull	26.20^{Ad}	8.72^{Ad}	14.67^{Af}	2.81^{Ab}
96	Heifer	16.80^{Ade}	8.43^{Ad}	7.68^{Bg}	0.73^{Ab}
	Mean	21.52	8.57	11.17	1.77
Overall mean		156.80	73.27	61.14	22.45

[1] For the same column, means followed by the same lower letter are not significantly different ($p<0.05$). Within the same column and for the same retention time, means followed by the same upper letter are not significantly different at ($p<0.05$) 0: In these time periods no seeds were recovered.

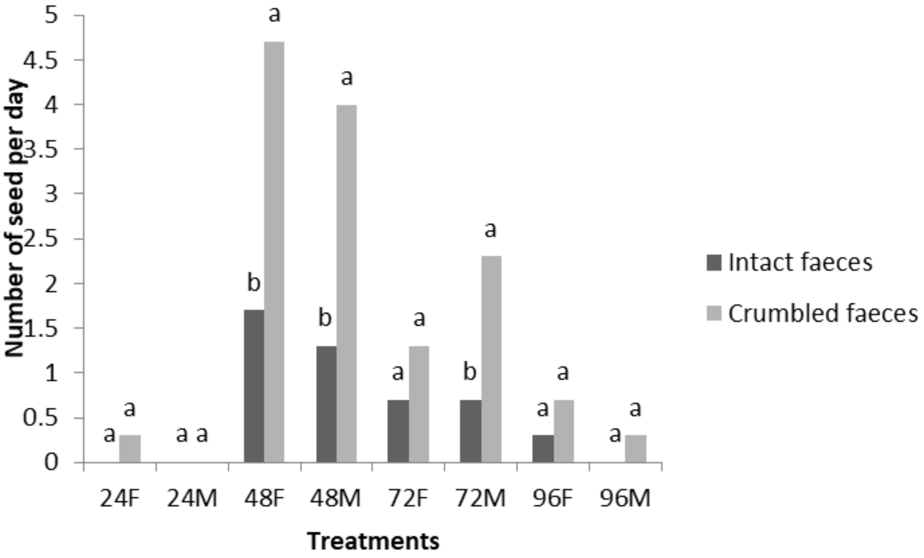

Figure 3. Number of *C. pubescens* seedlings per cattle(male (M) or female (F) emerged from intact and crumbled faeces on the soil surface collected after 24, 48, 72 and 96 h after ingestion. *Different letters indicate significant differences among number of *C. pubescens* seedlings per cattle emerged from intact and crumbled faeces on the soil surface collected after 24, 48, 72 and 96 h after ingestion (T- test; p=0.001).

the number fed. This confirms the result of Gardener et al. (1993b) that the fraction of rotten *C. pubescens* seeds is 89% after digestion by cattle. Cattle digested considerably higher amounts of *C. pubescens* seeds.

Overall germination percentage of seeds recovered from faeces (45.09%) was significantly higher ($P<0.05$)

compared to untreated seeds (31.00%) (Table 2 and Figure 2). This is in agreement with previous studies on frugivores gut treatment (Traveset, 1998; Razanamandranto et al., 2004). Before passage through the cattle the proportion of hard seeds (53.00%) in seed lot was significantly (P<0.05) higher than the amount (38.99%) after passage through cattle tract (Tables 3 and 4). Conversely, the amount of germinable seeds before passage through cattle tract was lower than that after passage (Tables 3 and 4). The mechanisms by which the digestive system stimulate germination could be through the separation of the seed from the shell, softening and scarification of the seed coat through mastication or action of acids and enzymes in saliva and stomach, action of faecal material present in the dung (moistening and fertilizer) (Traveset and Verdu, 2002). However anthelminthic applied may impact negatively seeds germination not only indirectly (reduced breakdown of faeces by dung beetles) but also directly through toxic effects (Eichberg et al., 2016). Also, the quantities of germinable and hard seeds recovered depended on the hard seeds content of the untreated seeds and the fraction of the hard seeds breaking down in the digestive system (Gardener et al., 1993a). The majority of the hard seeds of *C. pubescens* softened in the digestive tract. The breakdown of hard seeds followed by digestion explains the poor percentage of seeds recovered from faeces at the end of 96 hours (Gardener et al., 1993a). However, the results indicate that significant quantities of *C. pubescens* seeds can pass through the digestive tract of grazing cattle intact and almost 46.73% of these seeds will germinate within a month of wetting. These confirm the results of Lamphrey (1967), Souza and Júlio (2001) and Simão Neto and Jones (1987) who reported that, hard seeds often pass through the digestive tract of ruminants without being damaged. The survival of *C. pubescens* seeds after ingestion would serve two purposes: first, provide a cheap method of dispersing seeds and, second provide access to otherwise inaccessible land (Barrow and Havstad, 1992).

Seed retention time in the digestive tract is another important factor that affects the germination of seeds of some plant species (Traveset, 1998; Souza and Júlio, 2001). The number of seeds recovered after 72 h represented more than 91.00% of total seeds recovery. Barrow and Havstad (1992) found that about 95% of the recovered seeds of gelatin-encapsulated seeds fed to cattle passed through the steers within 72 h. It is generally believed that the longer the retention time, the more the seed coat will be exposed to mechanical abrasions, action of acids and enzymes and the better will be the germination of egested seeds (Razanamandranto et al., 2004). By contrast our result shows that the percentage of germinated seeds of *C. pubescens* did not increase, as the retention time increased from 48 h (47.83%) to 72 h (43.50%) (Table 2) which agrees with previous studies (Gardener et al., 1993a). This result is probably due to the fact that an increasing time of seed retention in the digestive tract had lowered fraction of germinable seeds and increased fraction of hard seeds (Table 4). The possible explanation could be that mechanical or chemical scarification of seeds with a long residence time in digestive tract may have allowed acids and enzymes to diffuse through the seed coat into the inner tissue, which eventually resulted in death of the embryo (Doucette et al., 2001). Some *C. pubescens* seedlings were observed in dung collected 48 to 72 h after injection. Seeds retention in the digestive tract of cattle for longer time may also induce germination during the digestive tract, followed by death of the seedlings (Stiles, 2000).

The total number of germinable seeds from young bull (253.00 seeds) and heifer (259.88 seeds) at the end of 96 h were not significantly different (p>0.05) (Table 4). The hypothesis of a difference in retention time for males and females due to their difference in body size which may affect the germination of seeds was not confirmed (Raymundo et al., 2018; Razanamandranto et al., 2004). According to the present results, both Lagune cattle sexes can potentially favor seeds dispersal of *C. pubescens* as sex did not significantly influence seeds recovery and seeds germination (p>0.05) (Figures 1 and 2).

The second part of the investigation observed the effect of being contained in dung for seedling emergence. Dung collected 2 and 3 days after ingestion of *C. pubescens* seeds had the highest concentration of viable (that is, hard and germinable) seeds. This dung was placed outdoors in a tray and left for 2 months. 100 g of fresh faeces collect each day during trial and broken-down on the soil surface produced an average of 7 plants per cattle, while those left intact on the soil surface produced 2 plants (p<0.05). So coprophageous insects that break-down the dung, trampling by grazing animals or another processes that disperse fresh manure, such as, heavy rain, may allow successful germination and emergence of *C. pubescens* seeds (Eichberg et al*.,* 2016; Mancilla-Leytón et al., 2012). However, when dungs are left intact for some months, the tight structure of dung act as a mechanical barrier and seedling emergence is thought to be unlikely (Grande et al*.,* 2013; Andrews, 1995). Additionally, seedling emergence in *C. pubescens* seeds retrieved from crumbled faeces at 48 h after ingestion was significantly higher (p<0.05) than the numbers of seedlings recovered at other times for all cattle (Figure 3).

The result demonstrates that break-down the dung and day of faeces collection had influence on seedling number. *C. pubescens* seeds are retained in cattle for 3 days and cattle can walk up to 14 km day^{-1} (Ghassali et al., 1998; Squires, 1981). Seeds can be disseminated over a large area, management of livestock to control spread of the plant is important. If the aim is to introduce *C. pubescens* to an area by passing seeds through cattle, the animals should be confined on that area for a minimum of 72 h after ingestion of seeds and preferably for at least 96 h to obtain a maximum recovery of seeds (Jolaosho et al., 2006). However, the endozoochorous dispersal involves cost and this cost is a sacrifice in the number of seeds surviving the passage through the digestive tract of cattle (Cosyns et al., 2005). This involves that a large number of seeds must have been consumed to compensate for the generally low seeds recovered and almost 46.73% of these seeds will germinate within a month of wetting.

For the third part of the investigation, the effect of soaking seeds with hot water and mechanical scarification were studied. Generally, it was observed that mechanical scarification was the method that had the highest percentage of germination (96.00%), germination speed (13.26 seeds/day), and the lowest mean germinated times values (1.75 days), followed by seeds immersed in hot water at 80°C for 2 to 4 min and seeds ingested by cattle. Some authors observed that mechanical scarification was the most effective way of breaking dormancy in seeds of *Leucaena leucocephala* and *Chrysophyllum abidum*, respectively (Aduradola et al., 2005; Duguma et al., 1998). Mechanical scarification has a positive effect on breaking dormancy because the damaging of lignified palisade cells after sandpapering permits water and oxygen to enter the cells (Yildiztugay et al., 2012). Mechanical scarification and duration of immersion in hot water affected the amount of germinable and hard seeds ($p<0.05$), compared with the germinable and hard seeds content of the untreated seeds. In the present study, increasing soaking time, increased the germination percentage and germination speed values and peaked at 4 min, while with longer time of exposure, the values of the two germination indices decreased. The highest of some germination indices of seeds immersed in hot water for 4 min might be attributed to the increased penetration of water and oxygen into the seeds. Gisachew and Scarisbrick (1999) and NFTA (1995) reported that treatment with hot water proved a very useful alternative for increasing the rate of germinable seeds. The highest rotten seeds were recorded in seeds immersed in hot water for 10 min (49.00%). The poor germination rates after immersing the seeds for 6, 8 and 10 min are probably a result of the death of embryo as caused by long time of exposure to hot water. So, a long time of exposure to hot water increased the amount of rotten seeds and decreased the amount of germinable seeds. Rincon et al. (2003) reported that soaking the seeds in hot water induced seeds germination; however, increasing the contact time of the seeds with hot water decreased seeds germination.

Endozoochory by Lagune cattle, mechanical scarification, hot water treatment at 80°C for 2 to 4 min can potentially favor seeds germination of *C. pubescens* and contribute to the rehabilitation of degraded grassland through enrichment planting. However, little is known about the relative costs and benefits of endozoochorous, mechanical scarification, and hot water treatment at 80°C for 2 to 4 minutes for enrichment planting. To understand from a plant's-eye-view, the role and relative importance of endozoochory compared with mechanical scarification and hot water treatment at 80°C for 2 to 4 min in rehabilitation of degraded grassland in Benin, we need to quantify the relative contribution of different techniques to later generation of the plants (Cosyns et al., 2005). This will require an integrate approach combining information from field observations with data concerning the region where seeds are deposited, in relation to cost of seeds lost from digestion for endozoochorous dispersal and consequences of seeds arriving on native species (Doucette et al., 2001; Gökbulak, 1998).

Conclusion

The findings suggest that both Lagune cattle sexes can potentially favor seeds dispersal of *C. pubescens* as sex did not significantly influence seeds recovery and seeds germination. The average number of seeds recovered from the cattle at the end of 96 h represented 7.65% of the number fed. Overall germination percentage of seeds recovered from faeces significantly increased compared to untreated seeds. The percentage of germinated *C. pubescens* seeds was not positively affected, as the retention time increased from 48 to 72 h. The result also demonstrates that break-down of the dung increased seedling number. It was observed that mechanical scarification was the method that had the highest percentage of germination (96.00%), followed by seeds immersed in hot water for 2 to 4 min at 80°C and seeds ingested by cattle. However, further studies are needed to understand the role, the relative cost and benefits of endozoochorous compared with the use of mechanical scarification using sand paper and hot water treatment at 80°C for 2 to 4 min in rehabilitation of degraded grassland in Benin.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Verification of integrated management of chickpea *Fusarium* wilt (*Fusarium oxysporum* f.sp. *ciceris*) in major chickpea growing areas of East Shewa Zone, Ethiopia

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Fusarium wilt is caused by _Fusarium oxysporum_ f.sp. _ciceris_, a major limiting chickpea productivity in Ethiopia. The present study was to validate the integrated management of chickpea _Fusarium_ wilt. The verifications were conducted in Adea, Lume and Gimbichu districts. The experiment was laid out as a factorial combination a farmer's field was used as replications. The management package consists of two varieties (Arerti and Habru), seed treatment with protective fungicides a rate of 2 g (a.i) kg/seed) and without and two seed bed type (raised and flat). There were three verifications per site. The pod borer management consists of timely insecticide application lambda cyhalothrin. The 2016 result showed significant variations among the treatment on _Fusarium_ wilt and hundreds of seed weight. The lowest _Fusarium_ wilt incidence (1.5%) was found on variety Arerti by raised bed and fungicide treated seed. The optimum yield (1747.5 kg/ha) obtained on flat plot with variety Habru use moisture in field, perhaps raised bed drain moisture. The correlation revealed that there was negative relationship between _Fusarium_ wilt and yield. While, in 2017 significant variation is on _Fusarium_ wilt and number of pods per plant. The highest _Fusarium_ wilt incidence (24%) was on variety Arerti by flat plot without fungicide. Whereas, the highest pod per plant (54.4%) was on variety Arerti by raised bed with fungicide treated. There was clear difference among the seasons on _Fusarium_ wilt incidences and yield. Thus, combining resistant cultivars by raised bed and fungicides treatment will reduce the _Fusarium_ wilt incidence and optimize planting time to obtain attainable yields and ploughing interval in severely infected field reduces primary sources of inoculum.

Key words: Chickpea, fungicides, *Fusarium* wilt, incidence, yield.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the second most important cool season food legume crop after common

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bean (*Phaseolus vulgaris* L.) followed by field pea (*Pisum sativum*) and third in production worldwide (Diapari et al., 2014). Currently, one of the widely cultivated crops at the global level on 13.5 million hectares of area with 13.1 million tons of grain legume is produced (FAOSTAT, 2014). The food legumes grown in different agro-ecological zones of the central, north, northwest, south and eastern highlands of Ethiopia (Merkuz et al., 2011). It is the main food legume in the northern and central highlands of Ethiopia (Keneni et al., 2012). The total area covered by chickpea in Ethiopia is estimated at 258,486.43 ha and from this a corresponding mean annual production of 472,611.4 tons of chickpea grain is produced (CSA, 2017).

An average chickpea yield in Ethiopia on field is usually below 2 t/ha although potential yield is 6 t/ha (Asnake, 2016). These huge gap between the potential yield were due to biotic and abiotic factors with current climate change scenario. This resulted from susceptibility of landraces to terminal drought, heat and no protection against weeds, diseases and insect pests (Asfaw et al. 1994). Although, more than 70 pathogens have been reported so far on chickpea from different parts of world and a few of them are currently recognized as significantly important to chickpea production (Pande et al., 2010).

One of the greatest biotic stress reducing potential yields in chickpea is chickpea *Fusarium* wilt caused by *Fusarium oxysporum* f. sp *ciceris* causing is a serious problem in rainfed area. Is one of the major asexual soil or seed borne disease of chickpea worldwide (Jalali and Chand, 1992; Kaiser et al., 1994). This fungus is pathogenic only on *Cicer* species (Jimenez-Díaz et al., 2015) of which chickpea is the only cultivated species.

Early wilting is reported to cause more yield loss (77-94%) than late wilting (24-65%), but seeds from late-wilted plants are lighter, rougher, and duller than those from healthy plants (Haware and Nene, 1980). In Ethiopia, about 30% yield loss of chickpea due to chickpea wilt has been reported (Meki et al., 2008).

According to Geletu et al. (1996) the disease caused yield loss of 50-80% in some farmers' fields. In addition to yield reduction, it also adversely affected the quality of grains by shrivelling the seed. The distribution and incidence of chickpea *Fusarium* wilt currently increasing annual loss estimated to 80% on North Shewa, West Gojjam (Personal observation).

Considering the nature of the damage and survival mechanism of the pathogen, management of the disease is difficult either through crop rotation or application of fungicides (Bakhash et al., 2007). The most practical and cost-effective method for management of chickpea wilt is the use resistant cultivars (Nene and Reddy, 1987). Resistant varieties can be economical and practicable method of disease management, but not be resistant to all the races prevalent in the area (Jimenez-Diaz et al., 1993; Meki et al., 2008). Merkuz et al. (2011) reported that *Fusarium* wilt incidence was reduced with different doses of green manure and dried plant residue. The recovery of the pathogens causing wilt/root rots decreased with delayed sowings (Seid et al., 1990). *Trichoderma* species are more effective when integrated with moderately resistant cultivars-controlled *Fusarium* wilt by 30 to 46% (Meki et al., 2008).

Fungicides like Thiram and Apron star offer a good protection against wilt (DZARC, 2005). Merkuz and Getachew (2012) reported that raised bed preparation, tolerant variety and optimum time of planting managed the wilt incidence. Effective microorganism, neem seed extracts and resistant variety had significant effects suppressive to *Fusarium* wilt. The EM-fortified compost tested in this study helped in controlling chickpea wilt (Negussie, 2012). Several studies have shown that soils containing beneficial microorganisms such as those found in the culture of effective microorganisms (EM) become suppressive to soil-borne diseases including *F. oxysporum* (Filion et al., 1999). Cultural practice like ploughing frequencies play great role in disturbing the pathogen life cycle, survival mechanism of pathogen. Verify integrated management for *Fusarium* wilt of chickpea on farmer's field in major chickpea growing regions and to attain optimum productivity of chickpea.

The objective of this study was to verify integrated management of chickpea *Fusarium* wilt and its yield components.

MATERIALS AND METHODS

The verification experiments were conducted in Adea, Lume and Gimbichu districts. The experiment was laid out as a factorial combination; farmer's field was used as replication with 10 m ×10 m plot sizes. The integrated management for *Fusarium* wilt consists of: two varieties with different reaction level moderately resistant to resistant type (Arerti and Habru), seed treatment with fungicides and control (42WS% Apron Star at rate of 2 g (a.i) kg/seed) and two seedbed preparation (raised bed and flat). In each districts there were three verifications per sites. The pod borer management consist of timely insecticide application (lambda cyhalothrin). Disease incidence (%) was collected on plot based after onset of disease symptoms. All disease incidence and yield and yield components data were subjected to analysis of variance (ANOVA) using SAS version 9.1. (SAS, 1997) using General Linear Model. Mean separation was based on the LSD at the 5% probability level.

RESULTS AND DISCUSSION

In 2016 cropping season, chickpea by raised bed and seed dressed with fungicide treatment combination showed statistically significant difference ($p<0.05$) for *Fusarium* wilt disease and hundreds of seed weight. The lowest *Fusarium* wilt incidence (1.5%) was recorded on variety Arerti by raised bed treatment and fungicide treated seed. Similarly, lowest *Fusarium* wilt incidence (3.5%) was on Habru by raised bed type while the

Table 1. Mean summary of seven traits recorded from on farm Integrated Pest Management verification chickpea tested in 2016 main cropping season at Lume and Gimbichu Woredas.

Treatment	FW (%)	PLHT (cm)	NPP	NSPlt	BMY (kg/ha)	HSW (g)	YLD (kg/ha)
Arerti + Flat + control	2.0	35.8	36.4	39.3	4662.5	26.83	1292.5
Arerti + Raised Bed + treated fungicides	1.5	40.0	38.0	38.6	4080.0	26.8	1512.5
Habru + Flat + control	5.0	39.3	29.9	33.5	3507.5	32.2	1747.5
Habru + Raised Bed+ treated fungicides	3.5	41.8	34.3	38.4	4285.0	30.4	1582.5
Grand Mean	3.0	39.2	34.6	37.4	4133.8	29.1	1533.7
CV (%)	19.2	19.9	13.9	12.96	25.6	3.1	24.1
LSD (0.05)	1.0	NS	NS	NS	NS	1.6	NS

FW= *Fusarium* wilt incidence (%), PLHT=plant height (cm), NPP: number of pods per plant, NSPlt= number of seeds per plant, BMY= biomass yield (kg/ha), HSW= hundred seed weight (g), YLD= grain yield (kg/ha).

Table 2. Correlation matrix (Pearson) between *Fusarium* wilt (FW) and yield and yield components trial planted in 2016 main cropping season at Lume and Gimbichu Woredas.

Variable	WRR	PLHT	NPP	NSPlt	BMY	HSW
PLHT	-0.201					
NPP	-0.409	0.550*				
NSPlt	-0.360	0.557*	0.988*			
BMY	-0.441	0.328	0.766*	0.778*		
HSW	0.699*	-0.335	-0.208	-0.132	-0.069	
YLD	-0.142	0.383	0.717*	0.741*	0.830*	0.233

FW= *Fusarium* wilt incidence (%), PLHT=plant height (cm), NPP: number of pods per plant, NSPlt= number of seeds per plant, BMY= biomass yield (kg/ha), HSW= hundred seed weight (g), YLD= grain yield (kg/ha).

highest *Fusarium* wilt incidence (5%) on variety Habru with flat plot (Table 1) implying that *Fusarium* wilt incidence is lower on raised bed than flat plot. The recovery of the pathogens causing wilt/root rots decreased with delayed sowings. However, early sowing (end of July) provided higher grain yields as compared to late sowings (Seid et al., 1990).

Although hypothetically higher yield was expected from chickpea cultivars with raise bed and fungicide treated seed (where *Fusarium* wilt occurrence was lower) though significant difference was not observed (Table 1). Lowest yield (15825 kg/ha) obtained on variety Habru by raised seed bed while the highest yield (17475 kg/ha) was harvested from Habru with flat practices plot (Table 1). *Fusarium* wilt of chickpea can be managed using resistant cultivars, adjusting sowing dates and fungicidal seed treatment (Navas-Cortes et al., 1998). However, the impact of variety and raised bed treatment did not affect significantly chickpea yield and yield components except hundred seed weight due to terminal stress.

The correlation matrix (Table 2) clearly revealed that there was negative relationship between *Fusarium* wilt and yield and yield components (yield, plant height, number of pods per plant, Number of seeds per pod, biomass yield) though weak in its magnitude. And hence consolidates theoretical justification of the objective of the study. Practically, however, the ANOVA output of yield failed to justify the fact that higher seed yield of chickpea variety will be obtained on plots with lower *Fusarium* wilt incidence. In this season, more yield on flat than raised bed which might be the stress as moisture drained from raised bed and flat plot retain moisture in the field as well as root growing depth to absorb water from depth. Planting of seeds at proper depth (10-12 cm) was helpful in reducing the disease incidence while shallow sown crop seemed to attract more disease (IAR, 1977). Whereas, variety by seed bed and fungicides treated seed combination showed statistically significant variations (p<0.05) for *Fusarium* wilt and number of pods per plant (Table 3) in 2017 season. The lowest *Fusarium* wilt incidences (8.0 and 8.6%) recorded on variety Habru by raised bed and fungicide treated seed followed by Arerti by raised bed type with seed treatment, respectively. The highest *Fusarium* wilt incidence (24.0%) obtained on combination of variety Arerti by flat plot and without fungicides treated seed. The highest pod per plant (54.4%) displayed on Arerti by raised bed with fungicide treated seed. Merkuz and Getachew (2012) reported that raised bed preparation, tolerant variety and optimum time of planting managed the wilt incidence and

Table 3. Mean summary of seven traits recorded from on farm Integrated Pest Management verification chickpea tested in 2017 main cropping season at three Woredas.

Treatment	WRR (%)	NPP	BMY (kg/ha)	HSW (g)	YLD (kg/ha)
Ararti + Flat+ Control	24.0	34.4	2800	14.8	800.0
Arerti + Raised Bed + Treated fungicides	8.6	54.4	3320	18.8	1360
Habru + Flat + Control	20.0	29.4	3452	14.8	1160
Habru + Raised Bed+ Treated fungicides	8.0	46.6	4342	21.0	1460
Mean	15.2	41.2	3478	17.4	1195
CV (%)	45.4	33.6	35.5	20.7	30.7
LSD (0.05)	10.39	19.09	170	9.16	506

FW= *Fusarium* wilt incidence (%), PLHT=plant height (cm), NPP: number of pods per plant, NSPlt= number of seeds per plant, BMY= biomass yield (kg/ha), HSW= hundred seed weight (g), YLD= grain yield (kg/ha).

reduce mortality of wilt.

Conclusion

Chickpea production and productivity were hindered due to *Fusarium* wilt disease among major biotic stress currently. The experiment was laid out as a factorial combination; a farmer's field was used as replication. The integrated *Fusarium* wilt management package consists two cultivars, seed treatment with and without fungicides and two seed type of bed preparation. The result verified that integrating tolerant cultivar by raised bed and fungicide treated seed at recommended rate will reduce the *Fusarium* wilt incidences and optimize the attainable yields in lack of resistant cultivar. The integrated pest management package should be practicable under farmer's practices and possible popularity especially high disease infestation area. Integrated disease management packages minimize the primary source of inoculums in soil as cultural control. Seed treatment improves germination and increases the plant stand uniformity. It needs more attention on frequency of ploughing to reduce inoculum source. Phenotyping of major races in the country needs focus. Seed inspection needs attention as diseases are seed borne while seed exchanging/seed system scheme.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Full Length Research Paper

Foliar application of zinc sulphate to improve yield and grain zinc content in wheat (*Triticum aestivum* L.)

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Foliar application is a promising agronomic strategy as it involves direct adsorption and loading of nutrients from leaf surface to phloem in comparatively far less quantity than soil applications. Present investigation entails the evaluation of most suitable treatment of zinc sulphate to improve growth and yield components of wheat. Significant increase in leaf length, leaf area, plant height, number of tillers, spike length, number of grains per spike, plant fresh weight, yield per plant, total soluble proteins and grain zinc content at 4 and 6 mM foliar treatments of zinc sulphate advocates 4 mM treatment more appropriate from economic perspective.

Key words: Wheat, zinc deficiency, foliar application.

INTRODUCTION

Zinc deficiency in soils, is an important constraint after nitrogen, phosphorus and potassium (Quijano-Guerta et al., 2002; Khan et al., 2008). Soil parent material, its weathering process, and frequent cultivation are the factors that reduce soil zinc availability (Almendros, 2008; Das, 2014). Zinc becomes unavailable due to its adsorption to oxides and hydroxides of Fe and Al and because of antagonistic effects of other divalent cations such as P (Lohry, 2007). Soil organic matter and temperature increases zinc availability as certain chelating agents are released on decomposition; while leaching and soil leveling erase top soil decreasing availability of zinc (Broadley et al., 2007; Kabir et al., 2014). Crops grown on zinc deficient soils exhibit chlorotic or necrotic spots on leaves, short stature of plants, uneven crop stand, delayed maturity, improperly developed fruits, decresed yield and low nutritional quality (Broadley et al., 2007; Alloway, 2008). This is because zinc is an important cofactors of more than 300 enzymes involved in different physiological pathways; maintains integrity of plasma membrane preventing plants from pests and insects and controls auxin levels in shoots and buds of plants. (Auld, 2001; Alloway, 2008; Nishizawa, 2015). Consumption of such zinc deficient crops as major staple food augments its inadequacy in humans and one-third of the world's population suffers from zinc deficiency (Hotz and Brown, 2004; WHO, 2009; Stein, 2010) evident in the form of impaired growth, slow healing of wounds, dermatitis, impaired appetite and anemia (Kiekens, 1995; Hambridge, 2000). Children under the age of five suffer from impaired immunity leading to diarrhea, pneumonia and malaria due to zinc deficiency (Hotz and Brown, 2004; Wessel and Brown, 2012). Zinc is also important in nucleic acids and protein

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synthesis and acts as neurotransmitter for being involved in cell signaling mechanism (Tapeiro and Tew, 2003; Hershfinkel et al., 2007). Zinc deficiency in humans is prominent in the areas where people mainly depend upon cereals such as wheat as their major staple food (Gibson, 2011; Cakmak, 2008). Wheat is an important staple food all over the world and it is cultivated over 240 million hectares worldwide (Wajid, 2002). Cereal grains should ideally contain 50 to 60 mg kg^{-1} zinc to fulfill the recommended daily dietary intake of 15 mg for young adults (WHO, 2009). Since, most of the existing wheat varieties are reported to contain zinc up to 29 mg kg^{-1} (Losak et al., 2011). Therefore, it is essential to develop some sustainable agronomic strategy to combat zinc deficiency (Mayer et al., 2008). In this context, foliar application is reported to be an uncomplicated, nominal and sustainable solution to address micronutrient malnutrition. (Graham, 2008; Voogt et al., 2013; Cakmak et al., 2010b). This method requires careful monitoring of crop genotype; suitable treatment and phonological stage; and soil and environmental conditions in order to get effective results (Shehu and Jamala, 2010; Fageria et al., 2011; Yuan et al., 2013). This method has the advantage of direct absorption of zinc through leaf surface and its prompt loading to phloem resulting in zinc traslocation along with photosynthetic assimilates towards developing grains (Boonchuay, et al., 2013; Shivay et al., 2015). Foliar applications are also reported to be effective where low soil temperature and moisture interferes with the micronutrient absorption (Rehman et al., 2014). Antagonistic effects of P on soil applied Zn can also be mitigated by zinc foliar applications, so that P can be applied at desired level to achieve better yield (Zhang et al., 2012). This method ascertains the economic effectiveness as it usually requires minimal amount of zinc carrier (Rengel et al., 1999; Voogt et al., 2013). This is because zinc is not wasted due to soil fixation and because of leaching or removal of top soil (Rehman et al., 2014). Zinc sulphate is preferably used as a zinc source for foliar applications as it is sparingly soluble, comparatively cheap and is immediately absorbed through the leaf surface; and its little amount (2 to 2.5 kg ha^{-1}) can give desirable results through foliar application (Das et al., 2014; Sarwar et al., 2015). Zinc is reported to be adsorbed and translocated quickly in first 6 to 12 h after foliar application (Doolette et al., 2018).

Keeping in mind economic and staple importance of wheat, the present research work focused to evaluate the zinc foliar treatments growth and yield components of wheat. Signifcant results on different parameters at 4 and 6 mM zinc sulphate treatments help evaluating the economic effectiveness of 4 mM treatment in order to get healthy produce of wheat along with enhanced zinc accumulation in its grains. Consequently, people belonging to poor resource settings who mainly depend on wheat as their daily dietary staple food may benefit with its improved nutritional quality along with fulfilling their daily requirement of zinc.

MATERIALS AND METHODS

Experimental material

Wheat seeds of seven cultivars (Punjab-2011, Faisalabad-2008, Aass-2011, Galaxy-2005, Sehar-2008, Chakwal-50 and Lasani-2006) were collected from Punjab Seed Corporation, Lahore, Pakistan and zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) in 0, 4, 6 and 8 mM (milimolar) concentrations.

Treatments

Three foliar applications of 0, 4, 6 and 8 mM zinc sulphate were given at vegetative phase at an interval of 15 days. Two foliar treatments were then given during grain filling, that is, at milk and dough stage, respectively.

Wheat sowing

This experiment was laid out in a randomized complete block design with four zinc sulphate treatments in two blocks at Seed Centre, University of the Punjab, Lahore in *rabi* season of 2015-2016. The soil of the experimental area was loamy with pH 8.5, EC 0.8, SOM 0.79%, P 1.2 mg kg^{-1} and K 55 mg kg^{-1}. High pH and low organic matter of the soil suggested that soil type used in this investigation could possibly be classified as zinc deficient. Experimental area for wheat sowing was well-prepared with seed beds in rows 15 cm apart. Seeds were sown by hand drill at a seed rate of 60 kg ha$^{–1}$.

Data collection at vegetative and at reproductive phase

Leaf length, leaf width and leaf area were recorded after one week of each zinc foliar application. Data on chlorophyll content of three randomly selected plants of each stand in the respective subplot were also recorded twice during vegetative phase of crop growth at an interval of 4 weeks; while days to flowering, days to anthesis and days to grain maturity were recorded when 50% of each plant stand exhibited the attribute. Plant height, spike length and number of grains per spike were recorded by randomly selecting plants from each stand.

Crop harvesting and data collection of yield components

The crop was harvested from each sub-plot separately at complete physiological maturity. Three of harvested plants were then randomly selected from each stand of zinc sulphate treatment to record respective plant fresh weight and dry weight. Spikes of the same plants were then cut and threshed manually to record grain yield per plant. Harvest index was obtained by calculating the ratio between the grain yield per plant and the dry weight of respective plants. 1000-grains weight was also recorded for each treatment.

Total soluble protein and grain zinc analysis

Total soluble protein content of grains from each of the four treatments (0, 4, 6 and 8mM) was analyzed by using Biuret method

Table 1. Type III sum of square, mean square and probability of F value of vegetative growth parameters and yield components of wheat at 0, 4, 6 and 8 mM foliar treatments of zinc sulphate.

Trait	Type III sum of square	Mean square	Probability of F
Leaf length (cm)	519.44	173.14	**0.00**
Leaf width (cm)	0.314	0.104	0.32
Leaf area $(cm)^2$	1325.10	441.70	**0.00**
Days to flowering	5.33	1.77	0.31
Days to anthesis	3.05	1.01	0.34
Days to maturity	4.05	1.35	0.24
Number of tillers	66.40	22.13	**0.00**
Spike length (cm)	32.14	10.71	**0.00**
Grains per spike	611.97	203.99	**0.00**
Plant height (cm)	730.44	243.50	**0.00**
1000-grain weight (g)	29.72	9.90	0.72
Plant fresh weight (g)	1172.73	390.91	**0.01**
Plant dry weight (g)	55.84	18.61	0.72
Yield per plant (g)	355.70	118.56	**0.00**
Harvest index	1.58	0.53	0.16
Chlorophyll content index	157.95	52.65	0.13
Total soluble proteins (mg g^{-1})	8.87	2.96	**0.00**
Grain zinc content (mg g^{-1})	0.02	0.01	**0.00**

of Roenson and Johnson (1961). Grain zinc content of samples from each stand was also analyzed by using the method of zinc analysis reviewed by Shar and Bhanger (2001).

Statistical analysis

The data were analyzed by PROC MIXED and PROC GLM in SAS statistical software package 9.3.1 (SAS Institute Inc., Cary, NC, 2001). Least square means of zinc sulphate treatments were calculated through two-way analysis of variance (ANOVA). Type III sum of squares were computed by PROC GLM and means were compared using Duncan's multiple range tests to rank the different treatments.

RESULTS

Improvement in vegetative growth of wheat plants was exhibited by statistically significant increase in leaf length, leaf area and plant height. Reproductive growth also exhibited significant increase in number of tillers, spike length, number of grains per spike, plant fresh weight, yield per plant, increase in total soluble proteins and grain zinc accumulation (Table 1).

Leaf length (cm), leaf width (cm) and leaf area (cm^2)

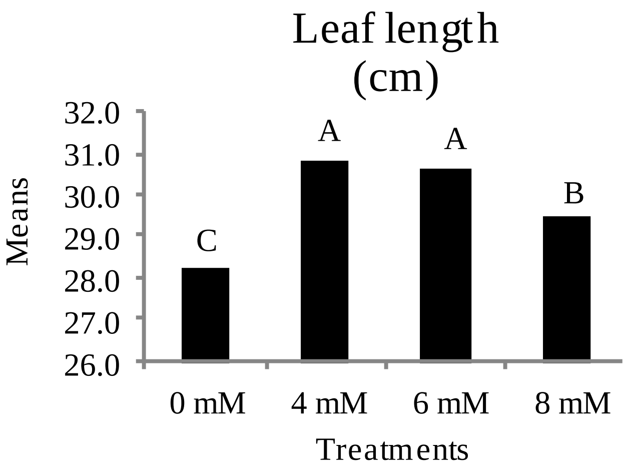
Least square means of observations exhibited maximum increase in leaf length (30.79 cm) at 4 mM. Leaf length increase was in the same range at 4 and 6 mM treatments (Graph 1). Leaf width did not exhibit significant increase and it was recorded to be in the same range at all the four treatments (Graph 2). A significant increase in leaf area was recorded at 4 mM treatment (33.50 cm^2) (Graph 3).

Days to flowering, days to anthesis, and days to maturity

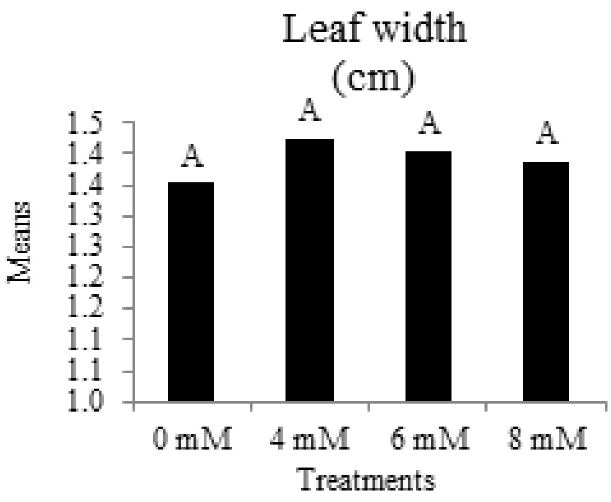
A non-significant change in days to flowering, days to anthesis and days to maturity was recorded at all the treatments. Maximum days to flowering (91.60) were observed at 8 mM; maximum days to anthesis (95.57) were exhibited at 6 mM; and maximum days to maturity (135.57) were exhibited at 6 mM (Graph 4, 5 and 6).

Number of tillers, spike length (cm) and number of grains per spike

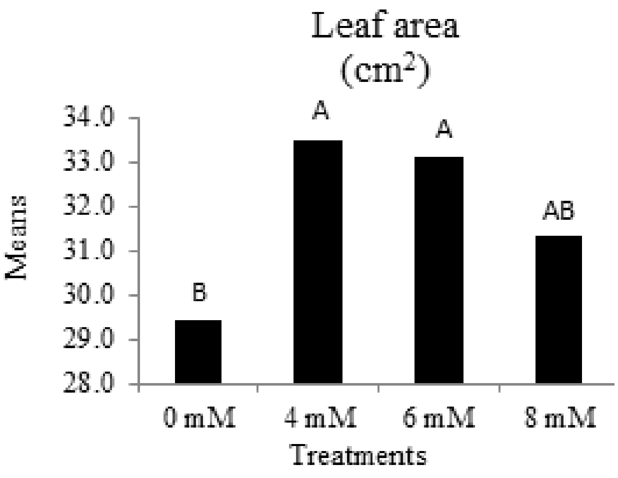
Maximum increase in number of tillers (5.61) was recorded at 4 mM; spike length exhibited significant increase at 6 mM (12.95 cm); and maximum number of grains (73.20) was observed at 4 mM (Graph 7). Spike length was in the same range at 4, 6 and 8 mM with a significant increase over 0 mM (Graph 8). Means and relative grouping of number of grains per spike based on DMRT also displayed that all the three treatments of zinc sulphate were in the same range with significant increase over 0 mM (Graph 9).



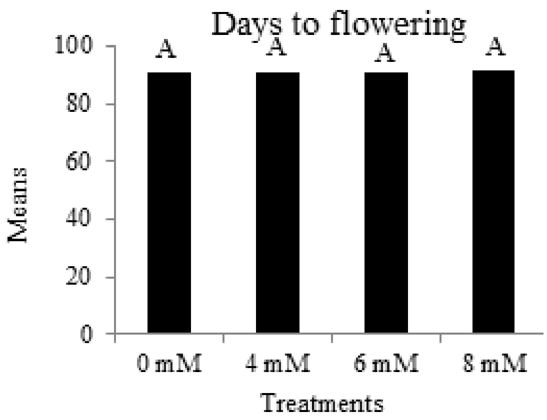
Graph 1. DMRT grouping of leaf length.



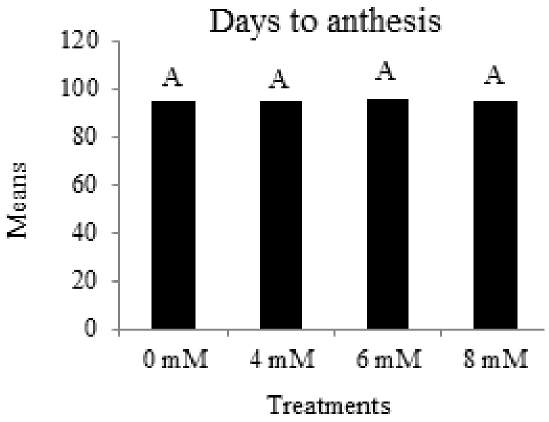
Graph 2. DMRT grouping of leaf width.



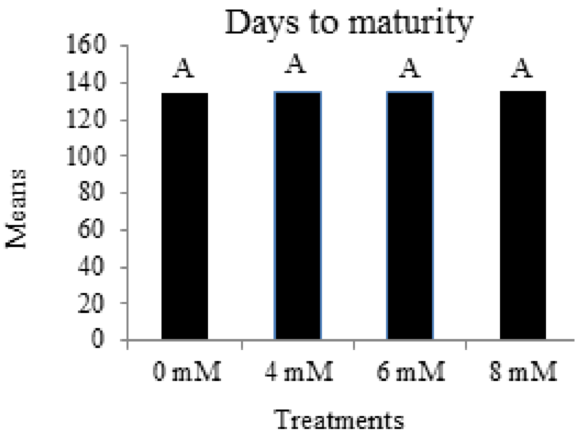
Graph 3. DMRT grouping of leaf area



Graph 4. DMRT grouping of days to flowering



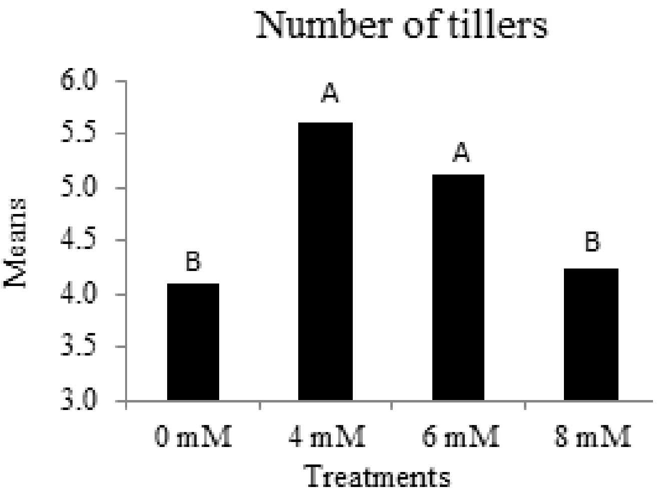
Graph 5. DMRT grouping of days to anthesis



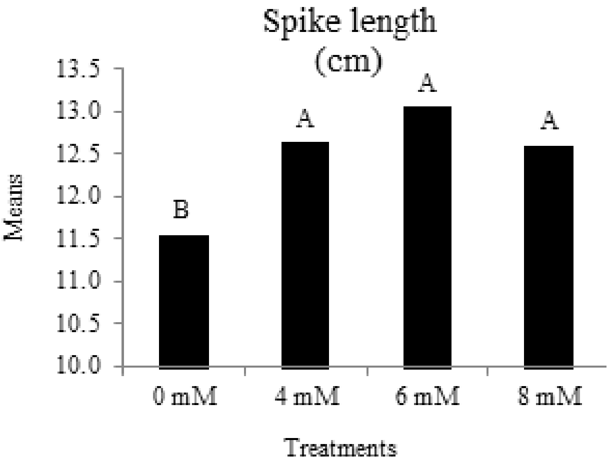
Graph 6. DMRT grouping of days to maturity

Plant fresh weight (g), plant height (cm) and 1000-grain weight (g)

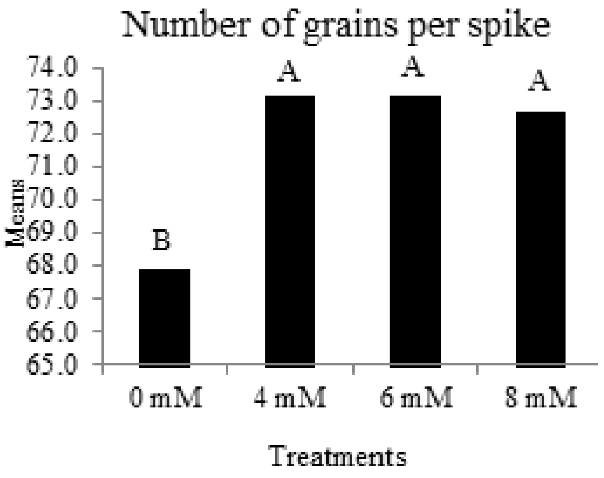
Significant increase in plant fresh weight (34.22 g) was



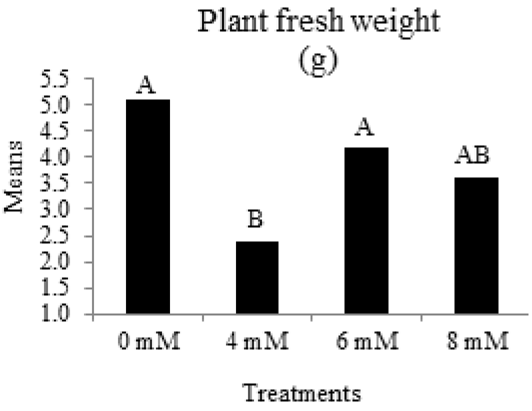
Graph 7. DMRT grouping of number of tillers.



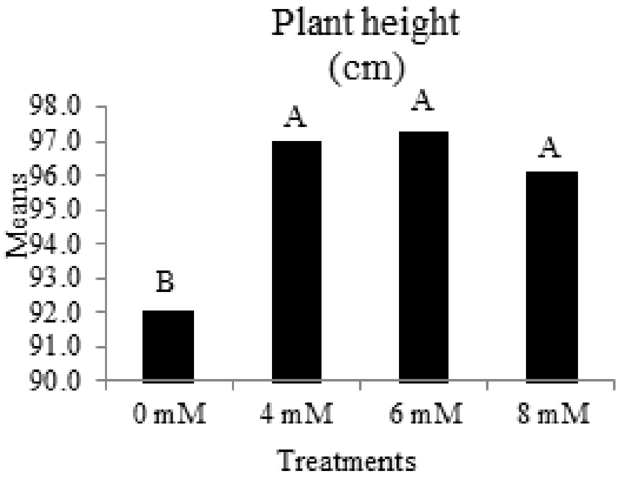
Graph 8. DMRT grouping of spike length.



Graph 9. DMRT grouping of grains per spike.



Graph 10. DMRT grouping of plant fresh weight

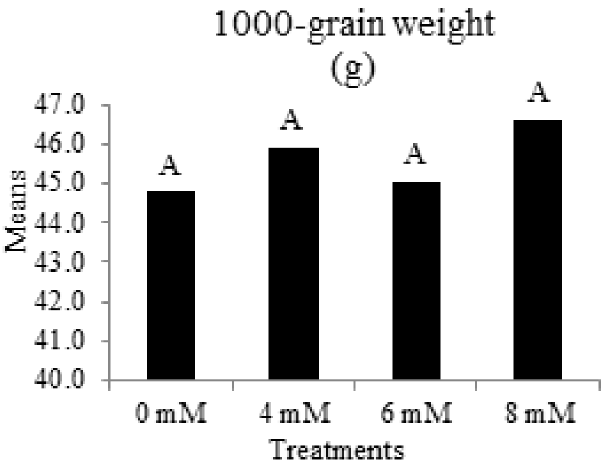


Graph 11. DMRT grouping of plant height.

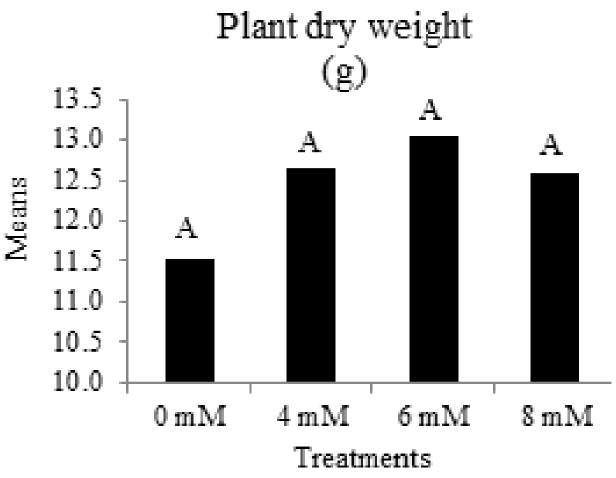
recorded at 4 mM zinc sulphate. Maximum increase (97.27 cm) in plant height was observed at 6 mM zinc sulphate treatment. Non-significant increase in 1000-grain weight was recorded at all the zinc sulphate treatments. Means and relative grouping of plant fresh weight also exhibited significantly higher range at 4 mM (Graph 10). Plant height also displayed significant increase at all foliar treatments of zinc sulphate over control (Graph 11). Non-significant increase in 1000-grain weight over control was also verified by means and their relative grouping based on DMRT in Graph 12.

Yield per plant (g), plant dry weight (g), and harvest index

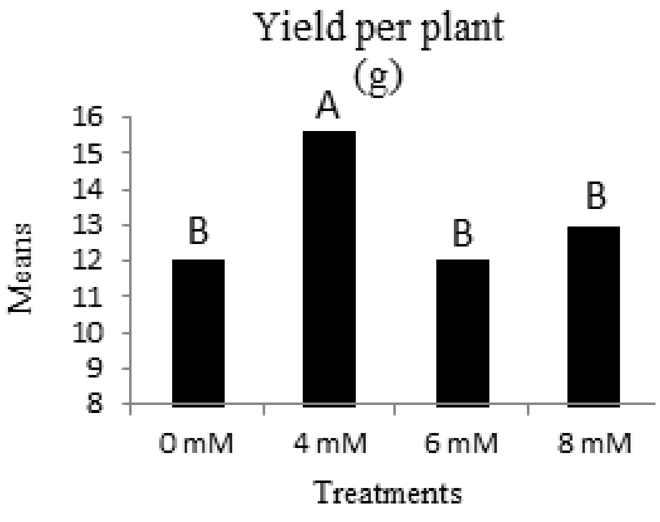
Maximum significant increase in yield per plant (15.60 g) was exhibited at 4 mM zinc sulphate treatment. Plant dry weight however, exhibited a non-significant increase at all



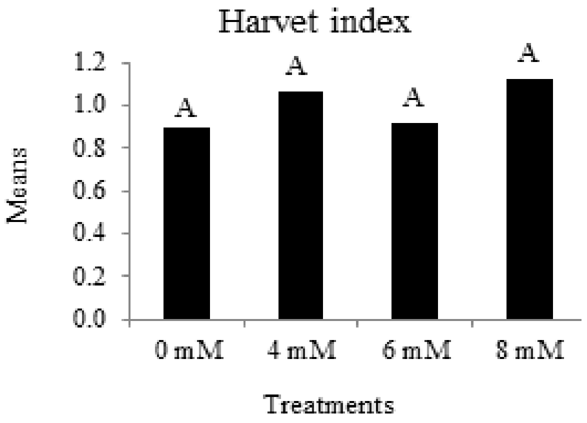
Graph 12. DMRT grouping of 1000-grain weight



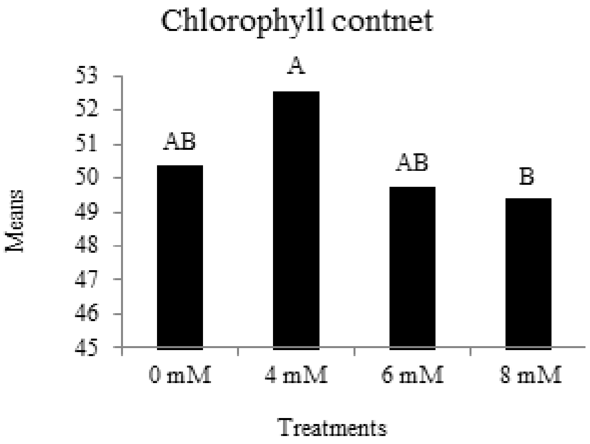
Graph 13. DMRT grouping of plant dry weight



Graph 14. DMRT grouping of yield per plant



Graph 15. DMRT grouping of harvest index

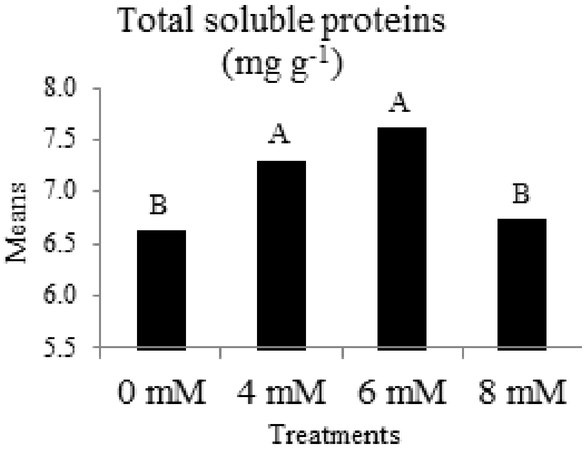


Graph 16. DMRT grouping of chlorophyll content.

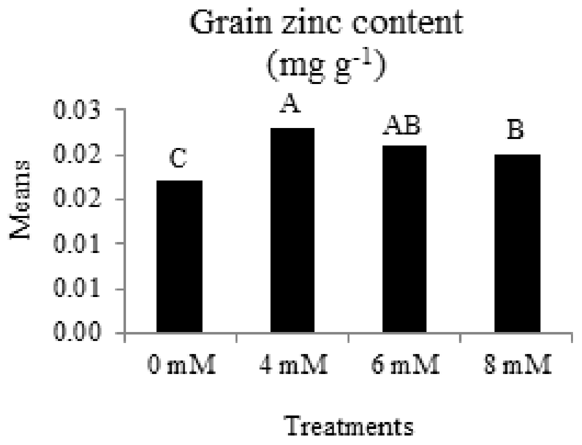
zinc sulphate foliar treatments with highest value (16.12 g) at 4 mM. The harvest index also exhibited non-significant increase at all zinc sulphate treatments and it was 1.12 at 8 mM. Increase in plant dry weight was in the same range at all zinc sulphate treatments (Graph 13). Graph 14 confirmed that yield per plant increased significantly at 4 mM. Similarly non-significant difference in harvest index by all treatments could be seen in Graph 15.

Chlorophyll content, total soluble proteins (mg g^{-1}) and grain zinc content (mg kg^{-1})

There was non-significant increase in chlorophyll content at all zinc sulphate treatments over control. A very slight increase (50.60) in chlorophyll content could be observed at 8 mM (Graph 16). Total soluble proteins exhibited significant increase over control with maximum value at 6 mM (7.61 mg g^{-1}). Grain zinc content was significantly increased up to 0.023 mg g^{-1} at 4 mM. Significant



Graph 17. DMRT grouping of total soluble proteins



Graph 18. DMRT grouping of grain zinc content.

increase in total soluble proteins was statistically in the same range at 4 and 6 mM treatments, whereas protein content at 0 and 8 mM treatment exhibited non-significant difference (Graph 17). Grain zinc content increase was in the same range at 4 and 6 mM treatment while, 0 and 8 mM treatments showed closely similar values of grain zinc content (Graph 18).

DISCUSSION

Wheat is reported to be a poor source of zinc having less than 20 mg kg^{-1} in most of the cultivars which should be more than 50 mg kg^{-1} dry weight of wheat grains (Zeidan et al., 2010). Zinc improves not only wheat growth and its yield components but also increases its water use efficiency (Singh, 2004). Dry matter accumulation and duration of reproductive growth is reported to be reduced at higher temperature during grain development and grain filling stage (Gibson and Paulsen, 1999). Zinc application may also combat with this yield limiting stress by increasing thermo-tolerance of the photosynthetic apparatus of wheat during high temperatures during ripening stage and maturation of wheat crop (Graham and McDonald, 2001). In the present experiment, many of the vegetative and yield components of wheat improved by foliar application of zinc. This could be related to the improved physiology of plants like photosynthesis, enhanced nutrient uptake, auxin activity, thermo-tolerance and water use efficiency.

The present observations on significant increase in leaf area index was in accordance with Khan et al. (2008) and Abdoli et al. (2014) who also reported an increase in leaf area index through zinc application. A minor reduction in flowering time at 4, 6 and 8 mM treatments of zinc sulphate supported a comparative lengthier grain filling duration. Findings of Abdoli et al. (2014) were in agreement with the present results as they also related increase in yield components and grain zinc components with reduced days to flowering. This led to lengthier grain filling duration which finally influenced the reproductive attributes of the crop.

The results on number of tillers, spike length and number of grains per spike were in great analogy with the work of Asad and Rafique (2000) and Hussain et al. (2005) who also reported increase in number of grains per spike and spike length by zinc application. Soleimani (2006) and Ali et al. (2009) reported a significant increase in number of grains per spike upon zinc application. Gomez-Beccera et al. (2010) explained that different cultivars behave differently in different locations, thus a combined effect of cultivar and treatment and particular agronomic managerial practices should be taken in to account while comparing the effect of different zinc treatments. The present results were analogous to the work of Arif et al. (2006), Jain and Dhama (2007) and Ranjbar and Bahmaniar (2007) who also noticed increase in grain yield by zinc application. Non-significant increase in plant dry weight of wheat cultivars was in great analogy with the work of Wang et al. (2012) who also did not notice any significant effects of zinc treatment in increasing biomass. The present results on non-significant increase in harvest index were found to be in agreement with the work of Hussain et al. (2005) and Abdoli et al. (2014) who also reported non-significant increase in harvest index by zinc treatments, while Imtiaz et al. (2003) and Ozkutlu et al. (2006) reported a reduction in harvest index owing to greater biomass. Jiang et al. (2013) and Aslam et al. (2014) reported a significant increase in chlorophyll with foliar application of 4 mM zinc sulphate. Potarzycki and Grzebisz (2009) also reported that zinc foliar application increased nitrogen uptake and protein quality which ultimately improved growth and yield components of the crop.

Bharti et al. (2013) observed an increase in methionine content with progressive application of zinc. Jiang et al.

(2013) also observed an increase in different enzymes activity in zinc treated plants. Liu et al. (2014), highlighted that increase in protein content and grain zinc content is mostly parallel to each other. Abdoli et al. (2014) reported that zinc foliar application increased grain zinc concentration from 9.4 to 19.7 mg kg^{-1}. Kutman et al. (2011) and Zhang et al. (2012) described that accumulation of zinc in vegetative tissue had a positive correlation with increase in grain zinc concentration up to 30 mg kg^{-1}. Abdoli et al. (2014) and Jiang et al. (2008) also noticed a three-fold increase in grain zinc content in comparison with control from 18.7 to 50 mg kg^{-1}. Up to 83.5% increase in grain zinc content was reported by Zou et al. (2012) who recorded almost consistent results over a wide range of 23 locations in seven different countries with their local cultivars and agronomic practices. Waters et al. (2009) and Liu et al. (2014) discussed the source and sink limitations in grain zinc accumulation. They emphasized that zinc translocation towards grains was not proved to be the limiting factor. Thus grains could accumulate quite high amounts of zinc by increasing its supply. Zhao (2011) also recommended that foliar application of zinc was preferable as it could increase yield attributes and grain zinc content up to 80%. Karim et al. (2012) reported a simultaneous increase in yield and grain zinc content in wheat. Cakmak et al. (2010b) reported that 10 mg kg^{-1} increase in grain zinc concentration was sufficient to combat zinc deficiency while foliar application increased grain zinc up to 20 mg kg^{-1}. This was helpful in achieving targeted levels of zinc in cereal grains. Zinc foliar application at early milk stage of grain filling is reported to significantly increase zinc concentration in wheat grain (Arif et al., 2006). Similarly Ozturk et al. (2006) also emphasized that frequent application of zinc at early milk stage (up to 10 or every third day) increased grain zinc content considerably along with progressive increase in in seed size and weight. Foliar application of zinc at milk and dough stage has also been reported to accumulate more zinc in grains than its application at earlier stages like stem elongation and booting stage by McCauley et al. (2009).

Conclusion

In current experiment on wheat most of the significant results were obtained at 4 mM (0.11%) and at 6 mM (0.17%) foliar treatments of zinc sulphate. Zinc sulphate was applied five times to wheat crop in 100 ml dose to each subplot of (2 × 2) ft. Grain zinc accumulation at 4mM (011%) zinc sulphate treatment was 0.023 mg g^{-1} in wheat experiment. This suggested that from its 100 g flour we may obtain 2.3 mg zinc. Use of an average of 300 g of this wheat will provide us with 6.9 mg zinc which may add well our daily need of zinc intake which is recommended to be in the range of 15 mg as per WHO (2009). These facts and figures may further help us to decide future perspectives of our research in terms of number and timely application of zinc foliar applications (Figures 1 to 6). Although, most of the significant results in this experiment were observed at 4 and 6 mM treatment of zinc sulphate however, for per hectare application 4 mM or 0.11% zinc sulphate may prove to be preferable from economic perspective.

Figure 1. Wheat sowing.

Figure 2. Seedling emergence.

Figure 3. Tillering stage.

Figure 4. Stem elongation stage.

Figure 5. Grain filling stage.

Figure 6. Mature crop of wheat.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Short-term effect of reducing conventional tillage intensity on some physical and chemical properties of volcanic soils in Rwanda

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Volcanic soils which comprise a minor portion worldwide are generally characterized by their high natural fertility yet susceptible to soil erosion due to their fragility. Regardless of which, the success in soil management to maintain its quality depends on the understanding of how the soil properties respond to its disturbance through tillage practices over time. This study was conducted in the highland region of Rwanda, Musanze District, Busogo Sector from December 2016 to June 2017, to evaluate the short term effect of conventional and reduced tillage on certain physical and chemical properties of volcanic soils. Bulk density (BD) and total porosity (TP) as induced soil compaction and aeration respectively were selected as soil physical properties. Soil pH, soil organic matter (SOM) and soil organic carbon (SOC) as generally used in soil fertility assessments and land suitability were selected as soil chemical properties. Soil samples were collected at the end of May 2017 from the plots laid out in RCBD replicated four times, then analyzed and data were subjected to ANOVA using GeniStat software. The results showed no significant difference in SOM and BD ($p>0.05$) while soil pH was significantly different ($p<0.05$) in these tillage systems. The results of this study evinced that reduced tillage is suitable in this region, since it is promising in the SOM enhancement. Evidences of this study will expose researchers and policy makers to new strategies to improve the soil structure stability, yet minimizing soil vulnerability in this highland region and countrywide.

Key words: Tillage, soil properties, soil disturbance, volcanic soils.

INTRODUCTION

Pedological surveys indicated that soils derived from Quaternary volcanic eruptions are widely distributed in the northwestern highlands of Rwanda hence the eight major volcanoes of Virunga Mountains are found in this region and around (Mizota and Chapelle, 1988). Soils that form in volcanic regions have andic soil properties

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and are classified as Andosols (World Reference Base WRB) or Andisols (Soil Taxonomy). These soils are often fragile characterized by lower bulk density, higher porosity, lower coarse fragment content and a disproportionate concentration of nutrients such as OM and total Nitrogen (N) in the upper 30 cm of the soil profile, hence are susceptible to physical disturbance such as compaction, landslides and erosion (Craigg and Howes 2007). This is worse in Rwanda due to the hilly nature of its topography interacted with excessive cultivation; these soils are particularly more vulnerable (Twagiramungu, 2006).

Despite their small proportion worldwide (1%), volcanic soils are highly fertile and support 10% of the world's population, including some of the highest human population densities (Neall, 2006; Astier et al., 2006). For Rwanda with the driving force of the economy being agriculture (Uwituze et al., 2017), soils derived from volcanic materials have a great importance as they support a large population of more than 445 inhabitants/km^2 (NISR, 2015) and much of the potatoes, cereals, and vegetables production are carried out in these soils. However, there is still a paucity of information about the nature, properties and proper management of these soils (Uwitonze et al., 2016).

Although Shoji et al. (1993) affirmed that farming practices change chemical and biological properties of volcanic soils; these soils continued to be under intensive cultivation which obviously led to their degradation (Uwitonze et al., 2016). Indeed, alteration of soil properties through disturbance mechanisms is not necessarily harmful, since all types of soils represent their unique nature and properties (Craigg and Howes, 2007; Valle and Carrasco, 2017). However, worthwhile soil disturbance standards or objectives must be based on measured and documented relationships between the degree of soil disturbance and subsequent soil properties response; studies designed to determine these relationships are commonly carried out as part of controlled and replicated studies for the purpose of determining threshold levels for detrimental soil disturbance exists (Craigg and Howes, 2007).

Tillage is an extreme form of soil disturbance that changes the soil properties and throughout the world many different tillage practices take place place. For many centuries, the conventional tillage system was used in agriculture to control the development of weeds, to incorporate crop residues into the soil, to recycle leached nutrients back to the surface, and to create adequate structure before planting (Oorts, 2006). With the introduction of herbicides the need for ploughing continued to be questioned and reduced tillage systems were adopted. Recently, tillage practices with minimum soil disturbance attracted much attention from researchers and policy communities due to their nature of increasing carbon storage, reducing erosion, increasing soil stability against compaction and overall soil structure (Palm et al., 2014; Schlüter et al., 2018)

Reduced tillage which consists of both superficial tillage and no tillage systems represents a relatively widely adopted soil management practice (Oorts, 2006). Thus, research on no-tillage oftentimes occurred within the context of conservation agriculture (Pittelkow et al., 2015). No wonder these terms may here in after be referred interchangeably (Quintero and Comerford, 2013; Lopez-Garrido et al., 2014; Pittelkow et al., 2015; Kabirigi et al., 2015; Schlüter et al., 2018). These reduced tillage systems have two main characteristics: The soil is not entirely turned over and the soil is always entirely or partially covered by residues; the main benefits of residue cover include improved soil water storage, enhanced soil organic matter content, nutrient recycling and protection against water and wind erosion (Oorts, 2006; Kabirigi et al., 2015). Although there is the potential for reduced tillage systems to contribute to impressive soil management practices, some recent reports argued that these benefits may not be as widely observed as previously thought or expected (Powlson et al., 2014; Palm et al., 2014; Brouder and Gomez-Macpherson, 2014; Pittelkow et al., 2015). Concerns expressed that reduced tillage systems can lead to excessive soil compaction which affects the crop growth (Steyn et al., 1995; Salem et al., 2015). Considering the harmful effects of soil compac¬tion in the field, the government of China, for example, started a campaign to pay allowance to the farmers who engage in conventional deep moldboard tillage practice instead of the conventional low moldboard tillage since 2009, encouraging farmers to solve the soil compaction by deep tillage (Ji et al., 2013). It is all clear that the amount of tillage to be employed to improve the soil properties is determined by many factors, these include: climatic conditions, soil types, crop rotation systems, etc. (Powlson et al., 2014; Kabirigi et al., 2015; Uwitonze et al., 2016). Thus, the effects induced by tillage also depend on different factors but generally the time of treatment (Da-Veiga et al., 2008).

On one hand, on a short-term scale, tillage operations mainly affect nutrient dynamics through altering of physical properties of the soil and by incorporating crop residues and mineral or organic fertilizers; and on a long-term scale, the short-term effects accumulate, thereby an additional system effect builds up (Pekrun et al., 2003). Mrabet et al. (2001) reported that reduced tillage systems increase soil organic matter (SOM) content in 3 to 5 years or more of continuous treatment, complemented with crop rotations and leguminous cropping, for the soils to become stabilized. On the other hand, McCauley et al. (2017) reported that tillage practices play a big part in soil pH change and Changes can occur within a season or last for decades.

Similarly, Da-Veiga et al. (2008) observed that change in Soil physical properties depends on tillage and time. Overall, the success in soil management to maintain the soil quality depends on the understanding of how the soil responds to tillage practices over time (Da-Veiga et al., 2008; Kiflu and Beyene, 2013). In recent decades, Rwanda adapted many policies and framework to improve soil quality and enhance soil conservation in which many of them did not even have a glimpse of success (Rushemuka et al., 2014). The arguments on conservation tillage systems (Kabirigi et al., 2015) and recent recommendation of their adaptation in this region (Uwitonze et al., 2016) left researchers a lot to debate. Thus, today's farmers in Rwanda are still unaware of the amount of tillage to be employed to improve the soil quality for seed bed preparation and cultivation (Kabirigi et al., 2015).

It is against the above background that this study aimed to employ the conventional tillage and reduce its intensity to evaluate the possible effects induced by tillage treatments on certain soil physical and chemical properties of volcanic soils in highland region of Rwanda on short-term basis. This enabled us to predict the amount of appropriate disturbance that improve properties of soil quality indicators, and consequently, minimizing overall the impact caused by soil mismanagement for long-term soil structure stability and agriculture prosperity as well.

MATERIALS AND METHODS

Study area description

The field experiment was conducted in UR-CAVM Busogo farm (University of Rwanda- College of Agriculture Animal Sciences and Veterinary Medicine)/Busogo Campus, Busogo Sector, Musanze District, Northern Province. Musanze is the most mountainous district of Rwanda as it contains the largest part of the Virunga National Park. Five of the eight volcanoes of the Virunga chain (Karisimbi being the highest peak of Rwanda with 4507 m, Bisoke, Sabyinyo, Gahinga and Muhabura) are within the district boundaries (Figure 1). Musanze District has many different agricultural possibilities as it is characterized by volcanic soil type, loose, well aerated and full of organic matter. According to National Soil Map of Rwanda (la Carte Pédologique du Rwanda) developed by Rwandan ministry of Agriculture, Livestock and Forestry in cooperation with Belgian government through Ghent University between 1981 and 2000; the soils of Busogo area as well as Rwanda's northwestern soils fall into the Andisol or Andosol type by soil taxonomy and this reflect to Virunga mountains. Busogo Sector is one of 15 Sectors of Musanze District in Northern Province of Rwanda which is made of 4 cells: Gisesero, Sahara, Kavumu and Nyagisozi respectively (Imerzoukene and Van-Ranst, 2002; NISR, 2015).

In general Busogo Sector has a mean altitude of 2300 m with the highest point being at 2800 m a.s.l. The climate has a mean temperature of 16.7°C and much rainfall comprising between 1400 and 1800 mm and is located at latitude of 1°33'26'' S and longitude of 29°32'39''E; Musanze-Rubavu road. Busogo Sector has 4 seasons divided as follows: light dry season from end-December to mid-February, heavy rainy season from mid-February to June, heavy dry season extending from June to end-August, and light rainy season from end-August to end-December. Volcanic soils of Busogo are very permeable with low depth on mountains and moderate depth in lower altitude characterized by Sandy loam texture. This kind of soil is subject to many erosion phenomena in the area of abrupt slope. According to Rwanda 4th Population and Housing Census, 2012 data; the population of Busogo sector is around 21,512 inhabitants, with the population density of 1069/km^2 and is the third sector with high population density after Muhoza and Cyuve respectively (NISR, 2015). Most people in Busogo Sector live in rural areas and they involve in agriculture; the main crops cultivated there are potatoes, maize, wheat, beans and vegetables (Uwituze et al., 2017).

Field experimental design

Before conducting experiment, the field of 25 m ×18 m has gone fallow for 5 years, and before that it was always under the intensive cultivation with seasonal crop rotation and carrying out experiments occasionally. Two types of tillage systems were used manually with the intention of leaving residue at the surface in one type of tillage system (Lopez-Garrido et al., 2014).

Experimental design was laid out in randomized complete block design (RCBD) with three treatments and four replications (Figure 2). Tillage treatments were; conventional tillage (T1) in which we dug the soil with a hoe up to more than 30 cm and the residues were fully incorporated, no residue was left at the surface and this technique is what is generally adapted for seed preparation by many farmers countrywide. The second was reduced tillage (T2) in which the soil was slightly disturbed and dug within 15 cm with the intention of leaving the residues on the soil surface, this system left in fact more than 50% of residues on the surface and the last treatment was control (T3), so the surface was left intact without any slight disturbance. The size of each plot was 5.0 m long and 5.0 m wide. A buffer zone of 0.50 m spacing was provided between plots with 1 m and 1.5 m space left at the Treatments' extremity and Blocks' extremity respectively. The cultivated plots were treated with cattle manure as one of the most affordable and commonly used means of soil fertilization countrywide equivalent to 12 t/ha. The composted cattle manure was applied by hand broadcasting prior to cultivation and fully incorporated in conventional tillage without being fully incorporated in reduced tillage. In the end of December, the activities of cultivation and sowing were over and the maize (*Zea mays*) was selected for the cultivated treatments. The space between rows was 1 and 0.5 m within rows, 2 seeds per stand were sown to give the population of 36000 seeds/ha, although after 8 weeks the second plant was removed to grow one plant per stand. The weeding was done manually with a hoe and hands after 2, 4, 8 and 12 weeks respectively. The corns with good stands in both tillage systems were harvested in mid-June two weeks after data collection for soil analysis.

Soil sampling

Since the primary purpose of our study was to determine how the soil properties were affected by each tillage type, the soil samples for determination of soil organic matter and soil organic carbon, soil pH, the soil bulk density and the total porosity were collected in the end of May in hope that some external pressure was acted on soil and became slightly stable.

Data for soil bulk density and total porosity as selected physical properties were collected after removing weeds, with the standard procedures adopted for recording the data for soil bulk density (Blake and Hartge, 1986); 12 undisturbed samples were taken from all plots by core samplers of nearly the same, transported directly to the laboratory and dried for 72 h at 105°C in the oven dry. Soil porosity was obtained from soil bulk density (Danielson and Sutherland, 1986).

Composite soil samples for SOM, SOC and soil pH analysis were collected in 0-30 cm of soil depth as discrete samples from each plot with a hand auger. The randomized quadrate sampling

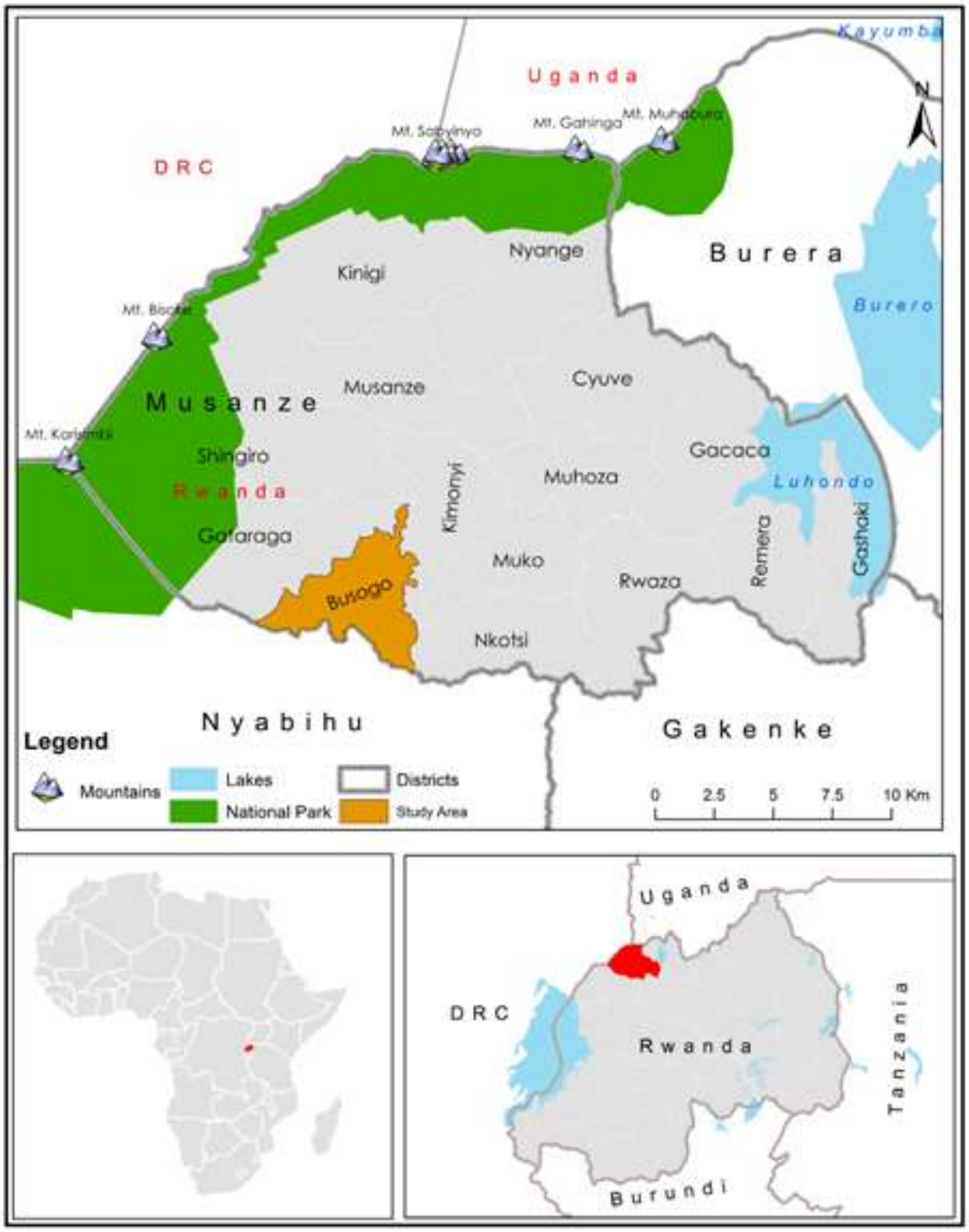


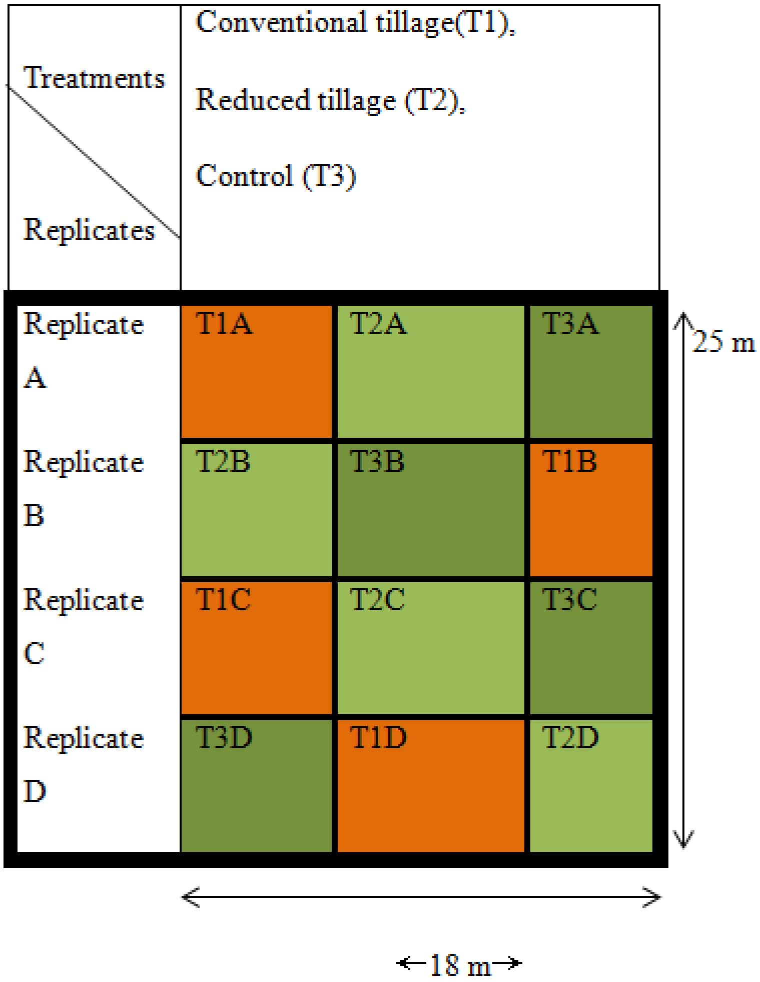
Figure 1. Musanze district administration map showing the position of Virunga Mountains and National Park, Busogo sector (the study area) and its location in Rwanda.

approach technique was used in which several sampling sites were chosen randomly within each plot, the samples from each plot were mixed in one plastic bag designating the plot and 12 plastic bags of mixed samples each were transported to the laboratory of soil science- UR-CAVM. They were dried for two weeks at room temperature, thereafter ground by mortar and pestle, and sieved by griddle mesh, divided into sub-samples depending on which parameters to be determined. SOM was determined from the soil sample ground to pass through 0.5 mm sieve, while soil pH was determined from the soil sample ground to pass through 2 mm sieve. They were all stored in sealed plastic containers at 25°C for laboratory analysis. Thereafter, the following parameters were analyzed and calculated according to the respective methods of their determination described in soil analysis.

Soil analysis

Determination of soil pH

Soil pH (H_2O) was determined on 2.5:1 water suspension of soil using a soil solution by the potentiometric method, using a glass



Replicate A	T1A	T2A	T3A
Replicate B	T2B	T3B	T1B
Replicate C	T1C	T2C	T3C
Replicate D	T3D	T1D	T2D

Figure 2. Diagram showing randomized complete block design used in experimental lay out.

electrode as outlined by (Okalebo et al., 2002).

Determination of soil bulk density and total porosity

Bulk density (BD) was measured using core method; by measuring soil mass of dry soil in the cylinder and volume of the cylinder according to the following equation (Blake and Hartge, 1986):

BD (g/cm^3) = weight of oven dry solid (g) / volume of soil (cm^3) (1)

Soil mass was measured after dried at 105°C for 72 h. Volume of the cylinder was obtained from ($R2\pi h$), Where R is the radius of the core cylinder, h is the height of the core cylinder; which coincides with the volume of the soil.

From this information, TP was calculated for each sample by using the method of TP from moisture loss; according to the following equation (Danielson and Sutherland, 1986):

TP (%) = 1 - BD / PD × 100 (2)

Where BD is the soil bulk density (g/cm^3) and PD is average particle density (2.65 g/cm^3).

Determination of soil organic matter and soil organic carbon

SOM content was determined using loss on ignition method (calcination) by destruction of OM in soil at an elevated temperature in a muffle furnace and measuring the weight loss (Nelson and Sommers, 1996). The weight of each crucible was weighed and a soil sample of 2 g sieved to pass through 0.5 mm mesh was placed on crucible and heated at 105°C in the oven machine for 3 h to remove the moisture and the weight was recorded; the soil sample was transferred to the furnace at 450°C for 3 h to extract organic matter. The sample was then cooled in desiccators and weighed. Organic matter content was calculated by the following equation (Nelson and Sommers, 1996):

OM (%) = [weight (g) at 105°C - weight (g) at 450°C] / [weight of soil sample (g) - moisture (g)] × 100% (3)

Then, from this equation OC was calculated as follow based on the assumption that OM contains 58% of OC (Nelson and Sommers, 1996):

$$OC (\%) = OM (\%) / 1.724 \quad (4)$$

Statistical analysis

The results from the collected data were statistically analyzed using analysis of variance (ANOVA) by GeniStat 14th edition software. The least significant difference (LSD) was used to test the significant difference between the means of different soil properties from three different treatments (at p = 0.05) (Habimana et al., 2015).

RESULTS AND DISCUSSION

All the results obtained from the treatments replicated four times each (Table 1). The mean values of the parameters which gave the true estimation of soil properties statuses were calculated and are summarized within the table.

Soil pH

The results showed that reduced tillage recorded a slightly lower pH value (5.700) relative to conventional tillage and naturally undisturbed (control) soil; 5.800 and 5.850 respectively (Table 1), yet the difference was statistically significant ($p<0.05$). The drop of soil pH in reduced tillage is probably due to the faster decomposition of the concentrated layer of organic residues lying at the surface with subsequent leaching of resultants organic acids induced by cattle manure application into mineral soil and due to the root exudates (Subbulakshmi et al., 2009). Our results showed that the conventional tillage have mixed the acidic layer with higher pH sub-surface layers (McCauley et al., 2017). Hulugalle and Weaver (2005) also reported that a decrease of soil pH is among the short-term events of soil properties which can result during the decomposition of residues due to the production of organic acids and microbial respiration; although some reports claim that tillage does not consistently increase or decrease soil pH without crop rotation throughout many years (Oorts, 2006). This was supported by López-Fando and Pardo (2009) who reported a lower pH in 20-30 cm depth of soil treated with no till than in conventional tillage after 5 years of rotating grey pea (*Pisum sativum* L.) and barley (*Hordeum vulgare* L.) and the attribution of these acidifying processes goes to mineralization of organic matter, nitrification of surface-applied N fertilizer and root exudation. Limousin and Tessier (2007) argued that the occurrence of these three phenomena in the upper layers of soil profile is reflected by the concentration of the leaf residues and higher roots density. Nevertheless, some previous studies reported a lower pH in No tillage compared to conventional tillage in 5 years of continuous corn treatment; without crop rotation (Blevins et al., 1977)

On the other hand, in his study Paul et al. (2003) demonstrated that 1.7 ton/acre residues in the top 1 inch can change pH by 0.02 units, these plants residues subsequently become SOM which has many benefits, the main benefit of SOM is that it buffers soil pH change (McCauley et al., 2017). These authors explained the process; SOM offers many negatively charged sites to bind with H+ in an acidic soil, or from which to release H+ in a basic soil, in both cases pushing soil solution towards neutral. Other controversial results were reported by Astier et al. (2006) in different tillage types treated with different green manure under maize. In their study, a significant difference in soil pH was observed in tillage systems treated by oat though conventional tillage recorded a lower pH than reduced tillage, on the other hand reduced tillage treated with Vetch recorded lower pH than conventional tillage but without a significant difference; however overall interaction of tillage with green manure was observed to lower the soil pH significantly than the bare tillage. Based on our results in this study, reduced tillage is not suitable since it tends to increase soil acidity, but based on what we reviewed there is a lot of controversy in literature. Thus whether the shift of soil pH in the short term and in the long term tillage practices still remain unclear and depends on many factors (Astier et al., 2006). So, much attention should be maintained on the regulations of soil pH in tillage systems.

Soil organic matter and soil organic carbon

One of the expected outcomes of this study from the beginning was the significant difference in Organic matter content that should be recorded by the treatments. But, this turned out not to be the case ($p>0.05$), despite SOM tended to be higher in reduced tillage (8.125%) than conventional tillage (7.500%), as well as the control treatment which recorded a value of 8.375%. Obviously, SOC also showed no significant difference after dividing by a common factor of 1.724 (Table 1). The record of higher Organic matter in reduced tillage relative to conventional tillage is probably due to the plant residue and litter left on the soil surface and to a slight disturbance of soil in the reduced tillage (Limousin and Tessier, 2007). Conventional tillage marked lower carbon content due to high rate of mineralization, faster litter and other buried organic residues decomposition and uneven redistribution caused by soil turnover (Astier et al., 2006; Wang, 2014; Ferrara et al., 2017).

Our results were consistent with the findings of previous studies which reported that conventional tillage operations result in more or less even distribution of SOM in topsoil, but in minimum disturbance the concentration of organic matter found in the superficial horizons of soil profile (Staley et al., 1988). Similar studies confirmed that reduced tillage practices often increases soil organic matter content while conventional tillage mix organic matter homogeneously, and also enhance its mineralization rate (Wang, 2014), although a significant

Table 1. T The soil properties values recorded in treatments after being experimented.

Types of tillage	Replicate	pH (water)	OC (%)	OM (%)	BD(g/cm^3)	TP (%)
T1	(A)	5.7	4.64	8.0	0.98	63.02
	(B)	5.8	4.35	7.5	0.96	63.78
	(C)	5.9	3.77	6.5	0.99	62.64
	(D)	5.8	4.64	8.0	0.99	62.64
Mean values		5.80	4.35	7.5	0.98	63.02
T2	(A)	5.6	4.64	8.0	1.03	61.13
	(B)	5.7	5.22	9.0	0.97	63.40
	(C)	5.8	4.64	8.0	0.98	63.02
	(D)	5.7	4.35	7.5	1.00	62.26
Mean values		5.70	4.71	8.12	0.995	62.45
CK	(A)	5.8	4.35	7.5	1.00	62.26
	(B)	5.9	5.22	9	1.11	58.11
	(C)	5.8	4.93	8.5	0.99	62.64
	(D)	5.9	4.93	8.5	1.05	60.38
Mean values		5.85	4.86	8.38	1.038	60.85
p value		0.027	0.244	0.244	0.208	0.208
l.s.d		0.099	0.674	1.162	0.072	2.715

T1: Treatment 1, Conventional tillage; T2: Treatment 2, Reduced tillage; CK: Control; l.s.d.: Least significant difference.

impact can take considerable years to occur (Mrabet et al., 2001; Ferrara et al., 2017). Continuous studies were suggesting that the increase in soil organic carbon associated with reduced tillage practices will continue for a long period of time (25 to 50 years) depending on climatic conditions, soil characteristics, and production management practices (Quintero and Comerford, 2013). A higher organic carbon in reduced tillage than conventional tillage without significant difference on short term basis was also reported by Tesfahunegn (2015). Still, our results were in line with that of Astier et al. (2006) who reported a higher organic carbon in reduced tillage relative to conventional tillage with non-significant difference in short term tillage under maize with different green manure treatments, similarly Quintero and Comerford (2013) reported a higher OM and OC content in reduced tillage than in conventional tillage; thus attributed these results to the remaining effects of oat cover crop roots on the organic matter content. In our case the remaining effects of grass roots induced by long fallow might be responsible, this is because intensive tillage systems accelerate the decomposition of soil organic matter in relation to vegetation, which allows organic carbon to rehabilitate. Kiflu and Beyene (2013) also emphasized that the roots of the grass and fungal hyphae in the grassland soils left intact are responsible for the higher organic matter accumulation. Tillage systems that reduce soil disturbance and residue incorporation was generally observed to increase SOM. Quintero and Comerford (2013) recommended these systems, emphasizing that due to higher OC concentration (and OM) recorded in tillage practices with minimum soil disturbance; then they can be used to restore soil carbon. This positive effect of reduced tillage practices on SOM and SOC contents not only reported by these authors since many studies observed the same benefits in different parts of the world (Curaqueo et al., 2010; Haddaway et al., 2016). Given that the purpose of this study was based on a limited time, it is not surprising that the results did not differ significantly (Mrabet et al., 2001).Still, these results reported here give the credits to reduced tillage for improving SOC and SOM, so they can be used by decision makers for future plans instead waiting the reports from studies that usually take a long period of time (Tesfahunegn, 2015).

Soil bulk density and total porosity

The results of BD showed a slight difference in the treatments yet statistically not significant (p >0.05). Undisturbed soil recorded a slightly higher bulk density (1.038 g/cm^3), followed by reduced tillage (0.995 g/cm^3) and conventional tillage (0.980 g/cm^3) (Table 1). The results clearly show that both undisturbed soil and reduced tillage were relatively more compact than conventional tillage. It was observed that bulk density of light textured soils increase in the tillage practices with the least soil disturbance in the top 200 mm of the soil when compared with the soils treated by conventional tillage systems (Steyn et al., 1995). The natural consolidation of intact soil and minimal disturbance of soils treated by reduced tillage should be condemned.

Our results were in line with that of Afzalinia and Zabihi (2014) who reported a non-statistical significant difference in BD between conventional tillage and reduced tillage at the end of corn growing season. These findings are also consistent with that of Manyiwa and Dikinya (2014) who reported that conventional tillage type can lead to a lower bulk density; which has significant effects on the soil's ability to allow easy water and solute movement and soil aeration and in crop and land management practices, yet their findings were not statistically significant. This is because compacted soils are associated with small pores of capillary size and therefore not penetrable by most roots (Steyn et al., 1995; Gbadamosi, 2013). Hence, would probably restrict water and air movement, as shown that reduced tillage and undisturbed soil are less porous compared to conventional tillage (Table 1).

However, some reports claim that tillage does not consistently affect bulk density since soil texture, aggregation, organic matter content and moisture conditions can induce the sensitivity of the soil to compaction (Steyn et al., 1995). Oorts (2006) argued that the bulk density in No till system remains fairly constant throughout the year while in Conventional tillage after the soil has been loosened by tillage, then soil bulk density will increase again by reconsolidation under the weight of the soil mass and machinery and due to the impact of raindrops and to drying/rewetting cycle. Still, this was supported by the findings of Afzalinia and Zabihi (2014) and Salem et al. (2015) who demonstrated that the bulk density in conventional tillage was lower than that of reduced tillage in the earlier months of corn growing season until it reaches its level of stability at the end of the growing season where there is no much significant difference between tillage treatments. On the other hand, "the total porosity is considered to be relatively low when it ranges from 13 to 27%", Pengthamkeerati et al. (2011); Manyiwa and Dikinya (2014) reported. Our study reflected extremely high porosity values in all treatments; reduced tillage (62.45%), undisturbed soil (60.85%) and conventional tillage (63.02%) with no significant difference ($p > 0.05$) (Table 1). This is because volcanic ash forms soils which are generally permeable, characterized by loose and well aerated physical status (Randy et al., 2008). The total porosity is smaller in the least disturbed soils due to higher bulk density but given the values of densities recorded by all tillage types it doesn't matter whether you choose any type of them (Manyiwa and Dikinya, 2014).

Based on the results of our study it is clear that reduced tillage practices and residue retention are promising in improving the soil properties such as SOM as one of the most determinant of soil fertility, productivity and the best of soil quality indicators (Kabirigi et al., 2015), not to mention more but they also enhance SOC stabilization in volcanic soils (Quintero and Comerford, 2013). Thus, the results from short term tillage studies can help us to predict the possible outcomes from them in future and help decision makers to set policies based on them rather than relying on the results from the long run basis which consume much time and cost (Tesfahunegn, 2015). Although our results were able to affirm that the volcanic soils properties improved by reduced tillage, further studies on their correlation with crops performance and yield are needed in order to fully trust them.

Conclusion

In general, our results of the study of reduced and conventional tillage short-term effect on certain chemical and physical properties in volcanic soil of Rwanda show that there is no significant impact of any tillage system on soil organic matter content and soil bulk density despite slightly difference between the values, but tillage systems affected soil pH significantly. Conventional tillage method was found to be better in improving the soil physical properties. This indicated that the soils under reduced tillage were relatively more compact than conventional tillage type but this difference in our study is negligible, since porosity was extremely high in both tillage systems. Conventional tillage system marked the lowest soil organic matter compared to reduced tillage. The results showed that the soil treated by reduced tillage became slightly acidic than conventional tillage; although the difference was statistically significant. Normally, Tillage with minimum disturbance of soil like reduced tillage in our study will improve Soil Organic Matter and Soil Organic Carbon content as the soil properties which indicate the soil fertility but it takes a certain time to accumulate. The results of this study confirmed that reduced tillage is promising and will be used by policy makers and all stakeholders in implementation of soil management policies. On the other hand, researchers are invited to take a part in this subject as it is still abstruse in Rwanda.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Production components and water efficiency of upland cotton cultivars under water deficit strategies

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The objective of this work was to study water deficits effect on different phenological phases in the production components and water efficiency of upland cotton cultivars. For this, an experiment was carried out at the Federal University of Campina Grande - UFCG, Pombal county Campus, Paraíba State, Brazil. Treatments were formed from a split-plot arrangement in which plots were 6 water deficit periods (P): (P1 = No deficit; P2 = Deficit in the initial growth stage; P3 = Deficit in the flower bud stage; P4 = Deficit in the flower stage; P5 = Deficit in the boll stage; and, P6 = Deficit in the open boll stage) and, the subplots, 2 upland cotton cultivars (C): (C1 = Brazil Seeds 286 and C2 = BRS 336), in randomized block design, with 4 replicates. Cultivars studied were more tolerant to water deficit in stages of initial growth, flower bud and open boll. Water deficit during flowers and bolls stages in upland cotton cultivars was the most detrimental to production components. Between cultivars tested, their behavior was similar only in cotton seed yield and water-use efficiency being BRS 286 higher than BRS 336 in other analyzed variables, except for mean open boll weight.

Key words: *Gossypium hirsutum* L. r. *latifolium* H., Hydric stress, agronomic variables.

INTRODUCTION

Cotton cultivation has great economic importance worldwide and it is also considered one of the main crops of great expression in the Brazilian economy. The cotton planted area in the 2016/17 season in the country was 930,400 ha, with lint production of 1,473,200 t in this harvest. While in the Northeast region, production was 361,000 t, in which the State of Paraíba contributed with 100 t of cotton lint in the 2016/17 season (Conab, 2017).

There is a marked presence of the genotype and environment interaction in the cotton crop, thus, a single cultivar cannot adapt to all cultivation regions of Brazil and it is important to identify the most appropriate

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cultivars for each ones (Carvalho et al., 1995). To this end, Araújo et al. (2013) stated that the success of a good agronomic performance of upland cotton will depend on the correct choice of the cultivar to be planted, as well as the environment and the cultural management.

It is necessary to know the agronomic and industrial characteristics of the cultivars commercialized in Brazil in order to ensure that producers will have technically and economically advantageous choices too. The same authors complement that cultivars that can adapt to different edaphoclimatic conditions are essential for an increase in the yield of any crop.

According to Shah et al. (2010), in the cultivation of upland cotton, the characterization of the stages of development of the crop by the chronological parameter results in extremely important variations regarding the real phenological stage when compared to different environments and/or years, as cultivation is highly influenced by the environment and the cultivar chosen, especially regarding thermal requirements. According to Araújo et al. (2013), knowledge regarding variations in the cotton plant during the development of these phenological stages is fundamental for the cultural management of the species.

According to Faggion et al. (2009), the recognition that water is an increasingly scarce natural resource imposes the need for more efficient production systems to ensure the sustainability of irrigated agriculture. Snowden et al. (2013) stated that the decrease in water availability may imply a need for changes and adaptations in irrigation strategies, since irrigation may be limited by low water availability in many regions. In this way, irrigation management is essential for the rational use of water in agricultural production to increase its efficiency. In the semiarid region, the cultivation of irrigated cotton is a good alternative for farmers, as it presents climatic characteristics that contribute to the production of good quality fibers and it can reach excellent yields (Brito et al., 2011).

However, research should seek to improve the irrigation management of cotton for high yields, high fiber quality and greater efficiency of water use by the crop (Zonta et al., 2015). The efficient use of water with adequate knowledge and the use of optimizing alternatives can contribute to increasing its availability, in this way reducing deficit problems caused by the increase in social demand in relation to environmental supply (Faggion et al., 2009).

It is important to study different cotton cultivars with water deficit applied on phenological stages in the semiarid region, since there may be cultivars that present different responses when subjected to water suppression in a certain stage of the cycle, which may lead to higher water-use efficiency and a more efficient crop production system. In addition, Zonta el al. (2015) stated that it is pertinent to test to what extent new cultivars respond to irrigation since many of them have been developed for the conditions of the Brazilian Cerrado and their cultivation coefficients may be underestimated for the semiarid conditions. Therefore, knowledge about the most tolerant stage of the cotton cycle for water stress can help in the decision of whether to use irrigation with controlled water deficit in some development stages, thus saving water without loss of yield, besides helping in the decision making of whether or not to use complementary irrigation during periods of drought.

The objective of this work was to study the effect of water deficit, applied on different phenological stages, in the production components and water efficiency of cultivars BRS 286 and BRS 336 of upland cotton, in order to relate the rational use of water for sustainable crop production in the semiarid region of Paraíba state, Brazil, and for the most appropriate irrigation management.

MATERIAL AND METHODS

The experiment was conducted under field conditions between June and December 2015 in the experimental area of the Center for Agricultural Science and Technology of the Federal University of Campina Grande, Campus of Pombal County, Paraíba State, Brazil, located in the following geographic coordinates: 06° 47’ 52” S, 37° 48’ 10” W and 175 m above mean sea level. The predominant climate of the region is hot semiarid (the BSh type), according to Köppen climate classification. The soil of the experimental area was classified as Fluvic Neo-soil (Santos et al., 2013), loamy sand texture (80% sand, 5.96% clay and 14.51% silt) and water tension curve of 15.49% (at 0.1 atm – Field Capacity - FC), 4.63% (at 15.0 atm – Permanent Wilting Point - PWP) with available water content (AWC) of 6.63% at the depth of 0–40 cm.

Fertilization was carried out according to the technical recommendations for the crop (Cavalcanti, 2008), based on the analysis of soil fertility as presented in Table 1, in the foundation by the application of 30 kg ha^{-1} of N, 40 kg ha^{-1} of P_2O_5 and 10 kg ha^{-1} of K_2O and in 2 covers, with the application of 30 kg ha^{-1} of N and 5 kg ha^{-1} of K_2O. Liming was not needed. Upland cotton cultivars were planted in single rows, spaced 1.0 m between rows x 0.10 m among plants.

The water used in the irrigation was of C_2S_1 salinity (low alkali and medium salinity hazard, with an electric conductivity - EC of 0.315 dSm^{-1}) and low sodium adsorption ratio (SAR = 1.78). Such water could be used for irrigation whenever there is a moderate degree of leaching and special care in the preparation of the soil. Water was applied by a localized irrigation system, with drip tapes and emitters spaced 0.10 m apart. Each treatment consisted of a lateral line, spaced from the other lines by 1 m with 6 m of length, each. Subsequently, after installation of the irrigation system and beginning of the experiment, a water distribution test was carried out in the field. Through this, the mean precipitation applied was determined as 8.86 mm h^{-1} and application efficiency (Ae) as 91%, according to Bernardo et al. (2008). Irrigations were carried out daily, always in the morning, based on the availability of soil water to plants. The replacement water volume was calculated considering the water evapotranspirated by the crop, which is represented as the difference between the soil water content in the field capacity and the current mean soil water content measured in the depths of 0.10, 0.20, 0.30 and 0.40 m, which were measured before irrigations. The current soil water content was determined by the time-domain reflectometry (TDR) method, using a Delta-T-PR2 probe introduced through access pipes installed in each treatment.

With the data of the current soil water content, using an Excel

Table 1. Chemical characteristics of the soil of the experimental area at different depths. Pombal county, Paraíba state, Brazil. 2015.

Depth	pH Water	OM (%)	P (mg 100 g^{-1})	Na$^+$	K$^+$	Ca^{2+}	Mg^{2+}
0-20 cm	6.79	1.16	51.5	0.14	0.42	4.28	1.40
20-40 cm	6.94	0.78	49.0	0.15	0.27	4.03	1.89

Source: Irrigation and Salinity Laboratory, UFCG, Campina Grande county, Paraíba state, Brazil.
pH = hidrogenionic potential; OM = organic matter.

Table 2. Detail of the deficit treatments. Pombal county, Paraíba state, Brazil. 2015.

Treatment	Period of application of the deficit	Beginning of the Deficit	Ending of the deficit	Total irrigation depth applied (La - mm)
No deficit (P1)	-	-	-	732.41
Deficit in the initial growth stage (P2)	22/Jul to 04/Aug	29 DAG	43 DAG	686.65
Deficit in the flower bud stage (P3)	03/Aug to 16/Aug	40 DAG	54 DAG	608.39
Deficit in the flower stage (P4)	18/Aug to 31/Aug	54 DAG	68 DAG	603.53
Deficit in the boll stage (P5)	26/Aug to 08/Sep	62 DAG	76 DAG	610.85
Deficit in the open boll stage (P6)	03/Oct to 16/Oct	100 DAG	114 DAG	649.67

(P1), (P6) = treatments designation; DAG = days after germination.

spreadsheet in which the daily values of the current soil water content and the availability of water to plants were recorded, the depth for the replacement of water and the time of irrigation were calculated for the treatments, which were the basis for the determination of the net and gross irrigation depth (NID and GID), according to Mantovani et al. (2009).

Treatments were formed from a split-plot arrangement in which the plots were 6 water deficit periods (P): (P1 = No deficit; P2 = Deficit in the initial growth stage; P3 = Deficit in the flower bud stage; P4 = Deficit in the flower stage; P5 = Deficit in the boll stage; and, P6 = Deficit in the open boll stage) and, the subplots, 2 upland cotton cultivars (C): (C1 = Brazil Seeds 286 and C2 = BRS 336), in randomized block design, with 4 replicates, amounting to 48 experimental subplots. Each period of water deficit consisted of 14 days without irrigation in the predetermined phenological stage, according to Table 2. After this period, the plants had normal irrigation until the end of the cycle. The total irrigation depth applied for each treatment was also presented in Table 2. The necessary phytosanitary treatments were carried out when the first injuries and symptoms of pests and diseases appeared, as well as crop treatments for weed control.

The number of open bolls per plant (NOBP_dimensionless) was determined by counting its total per plant in the subplot. The mean open boll weight (MOBW_g) and fiber percentage (F_%) were respectively determined on the subplot by the mean cotton seed weight (CSyield_kg ha^{-1}) of the 20 open bolls collected in the standard sample at the time of harvest and by weighing the lint/fiber after processing, which result in the percentage rate between total cotton lint weight (CLyield_kg ha^{-1}) and total CSyield in that sample.

CSyield was determined by harvesting and weighing the cotton seed production of the useful area of each subplot, extrapolating per hectare (kg ha^{-}1). Mean CLyield was calculated by multiplying the mean CSyield by F. Water-use efficiency (WUE_kg m^3) or water yield was defined as the ratio between the CSyield found (Ya) (kg ha^{-1}) and the total water used during the cycle (La) (m^3 ha^{-1}) for each treatment considered in the study (Geerts and Raes, 2009).

The obtained data were subjected to analysis of variance through the F-test and the means of the factor levels or treatments, both qualitative, were compared by the Tukey test at 5% of probability using the statistical program SISVAR (Ferreira, 2011).

RESULTS AND DISCUSSION

According to Amaral and Silva (2008), the soil moisture profiles were evaluated in this layer during 126 days in all treatments of water deficit periods as presented in Figure 1, comparing them to the water content in the FC and PWP averages of soil of experimental area, because the higher concentration of cotton roots is in the 0.0 to 0.40 m depth layer. It can be observed that soil moisture in all treatments of each water deficit period was very close to the PWP, which increased during the period of application of the deficit and remained in approximately 50% of the AWC after this application. The deficit treatment applied in the open boll stage presented the same behavior of the irrigated treatment until a little before the application of the deficit period as presented in Figure 1.

According to Sun et al. (2015), tolerance to water stress depends on the plant growth stage and, when water deficit occurs at critical stages such as the reproductive stage, plant growth and development may be affected. Thus, it is very likely that the metabolic and physiological functions of the plants have been severely affected in this study.

The deficit Periods (P) affected the NOBP, MOBW, CSyield, CLyield, F and WUE ($p \leq 1\%$). Cultivar (C) influenced the NOBP, MOBW, CSyield and F ($p \leq 1\%$). Regarding the interaction (P x C), there was effect only for MOBW ($p \leq 1\%$) as can be seen in Table 3.

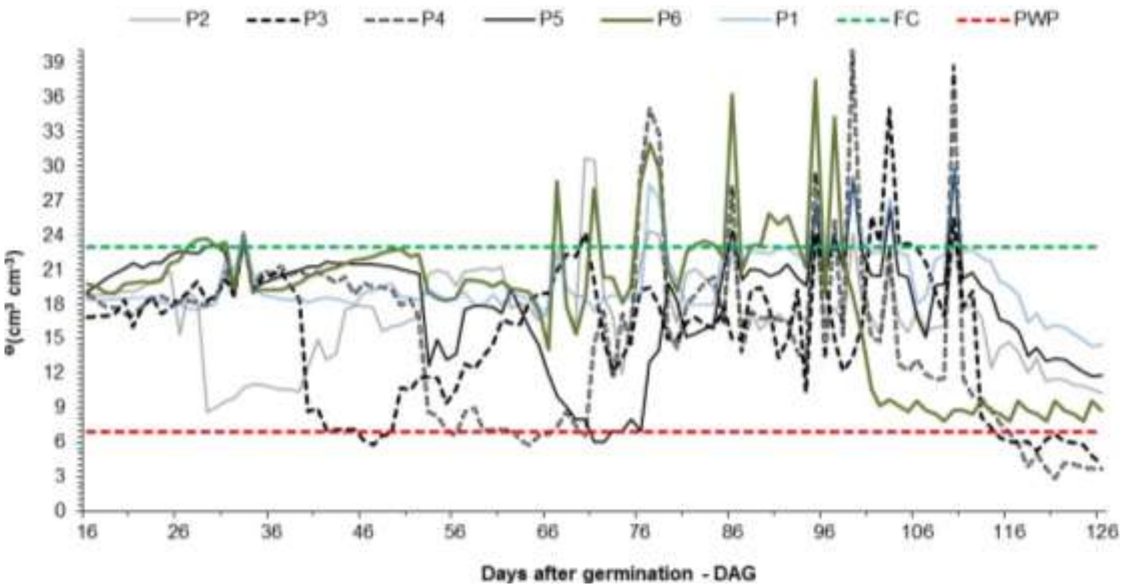

Figure 1. Variation of soil water content on the different water deficit treatments along experimental period. Pombal county, Paraíba state, Brazil. 2015.

Table 3. Summary of the analysis of variance for production components and water efficiency variables of two upland cotton cultivars under different water deficit strategies in the phenological stages. Pombal county, Paraíba state, Brazil. (2015).

SV		**NOBP**	**MOBW**	**CSyield**	**CLyield**	**F**	**WUE**
	DF	**MS**					
Blocks	3	2.48	0.01	180438.55	24812.51	2.77	0.0042
Deficit periods (P)	5	115.82**	2.32**	11515815.40**	1950556.14**	8.65**	0.1796**
Error 1	15	3.66	0.12	565194.48	97831.48	0.84	0.0135
Cultivar (C)	1	56.87**	9.72**	184973.56^{ns}	590693.37**	403.68**	0.0056^{ns}
P × C	5	2.40^{ns}	0.53**	314496.20^{ns}	27220.60^{ns}	4.32^{ns}	0.0071^{ns}
Error 2	18	2.33	0.07	122054.23	19502.74	0.89	0.0028
Total	47						
General mean		8.72	6.23	2971.01	1235.93	41.57	0.45
CV 1 (%)		21.95	5.70	25.30	25.31	2.21	25.84
CV 2 (%)		17.50	4.29	11.76	11.30	2.27	11.87

^{ns}, ** and *: not significant and significant at $p \leq 0.01$ and $p \leq 0.05$, respectively (F-Test). MS = Mean squares; CV = coefficient of variation.

Bezerra et al. (2003), when studying the effect of soil water deficit on the cotton lint yield of the upland cotton cultivar BRS 201, have reported that yield was affected by the water deficit in the various crop development stages, with a significance level at 1% probability. Zonta et al. (2015) have also found significance at 1% probability for the studied factors when evaluating the effect of irrigation on cotton lint quality and yield; in addition, Zonta et al. (2017) also have found significant differences at 1% probability for the factors studied when evaluating the response of cotton to water deficit in different stages of the crop cycle. Relative to the effect of the water deficit strategies studied (deficit periods), upland cotton showed a tendency to decrease the NOBP when the plants were subjected to water deficit in different phenological stages, which was present when irrigation was stopped in the stages of P2, P3, P4 and P5, but not in P6, when this decrease was smaller as presented in Figure 2A.

Mean NOBP decreased in 44.44, 43.13, 58.16, 75.16 and 37.25%, respectively, in relation to the treatment without water deficit (P1) as shown in Figure 2A. This is probably because the water deficit caused a decrease in flower buds, flower abortion and/or shedding of bolls,

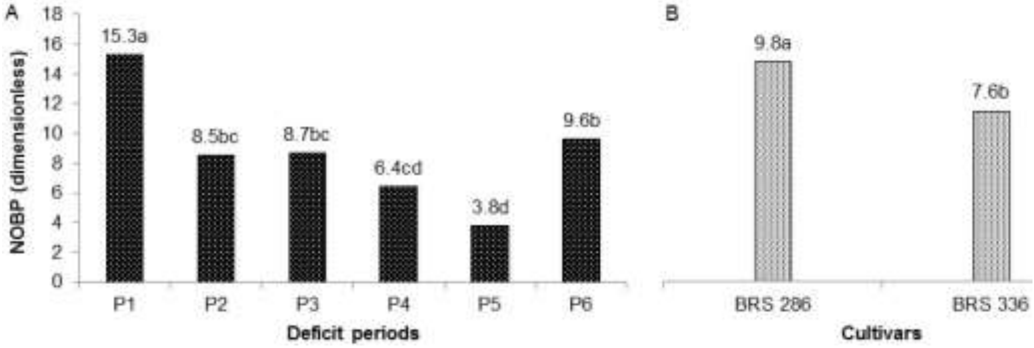

Figure 2. Mean number of open bolls per plant of two upland cotton cultivars under different water deficit strategies in the phenological phases (A. Deficit periods; B. Cultivars). Pombal county, Paraíba state, Brazil. 2015. Same letters in the factors (A and B) indicate no significant difference among means (Tukey, p<0.05).

resulting in lower NOBP. According to Zonta et al. (2017), the water deficit applied in the initial growth (P2) and open boll (P6) stages had the least effect on the NOB per meter, since either the plant did not yet have reproductive structures or it already had most of its bolls formed, as the stage of formation of open bolls (maturation stage) is tolerant to water stress (Jalota et al., 2006), which are similar to the results obtained in this study.

These results also are similar to those reported by Silva et al. (1998), who studied the effect of water stress on the phenology and some technological characteristics of the upland cotton fiber CNPA 6H and by Ünlü et al. (2011), who stated in their studies that deficit irrigation caused a significant decrease in the NOBP. As well as they were the same of Almeida et al. (2017), when studying the effect of water deficit on upland cotton production, stated that there was a decrease in the NOBP in the water deficit periods, and Zonta et al. (2017), who stated that an important characteristic related to yield is the NOBP, since the higher retention of open bolls will represent higher yield. The latter authors also stated that the NOBP was affected by water deficits and the best results were obtained by the treatments without water restriction, followed by treatments with water restriction in the stages of initial growth (P2) and first boll opening (P6) and lastly the worst results were in the stages of appearance of the first flower bud (P3), the first flower (P4) and the first boll (P5) (Zonta et al. 2017), as observed in this work.

The stage of flowering (flower) and fruiting (boll) (P4 and P5) were the less tolerant to soil water deficit as presented in Figure 2A, whose result was similar to Souza et al. (1997), who found decreases of 23 and 53% in the NOBP on the fourteenth day of stress when studying the influence of soil water saturation on the physiology of cotton CNPA 7H. According to Beltrão (2006), these stages are triggered from the flowering to the opening of the bolls during a variable period, after which fiber is obtained, which is considered the main product of cotton.

Snowden et al. (2014) also observed decreases of 60% in the NOBP when comparing the treatments with water deficit for 3 weeks after the flowering and control treatment, with similar results to those found in this study in the water deficit treatment in the stage of appearance of the first open boll (P6). Gwathmey et al. (2011) stated that water deficit at the beginning of flowering tends to increase the shedding of floral buds, whereas water deficit at the end of it reduces the rate of flowering and retention of bolls, which also is similar to the results obtained in this study.

Regarding the cultivar factor, cultivar BRS 336 had a lower value for the NOBP in relation to cultivar BRS 286, with mean values of 7.63 and 9.81 NOBP, respectively as presented in Figure 2B. According to Iqbal et al. (2010), Baloch et al. (2011) and Niu et al. (2013), tolerance to abiotic stress, including drought tolerance, varies according to genotype.

Within the effect of the cultivar in the water deficit strategies (deficit periods) in MOBW, cultivars BRS 286 and BRS 336 differed statistically among all water deficit periods except in P5. Overall, the MOBW of cultivar BRS 336 was less affected than BRS 286 by the applied water deficits as shown in Figure 3A.

Related to the deficit periods in the cultivars for MOBW, it can be observed that cultivar BRS 286 showed the highest MOBW values in the deficit periods P1 and P2 (control and water deficit in the initial growth stage); in turn, cultivar BRS 336 presented the same behavior as presented in Figure 3B. In general, for both cultivars, MOBW decreased as the deficit periods were applied in the different phenological stages of the cotton plant, but water deficit was more restrictive after the flower bud (P3) stage as can be seen in Figure 3B.

Therefore, cultivars BRS 286 and BRS 336 presented differences between each other in most of the studied treatments and regarding the variety standards, which is 5.5 to 6.0 g for BRS 286 (Silva Filho et al., 2008) and 6.6 g for BRS 336 (Morello et al., 2011) and some treatments had MOBW above or below these ones as presented in Figure 3 A and B. Silva et al. (1998), studied the effect of

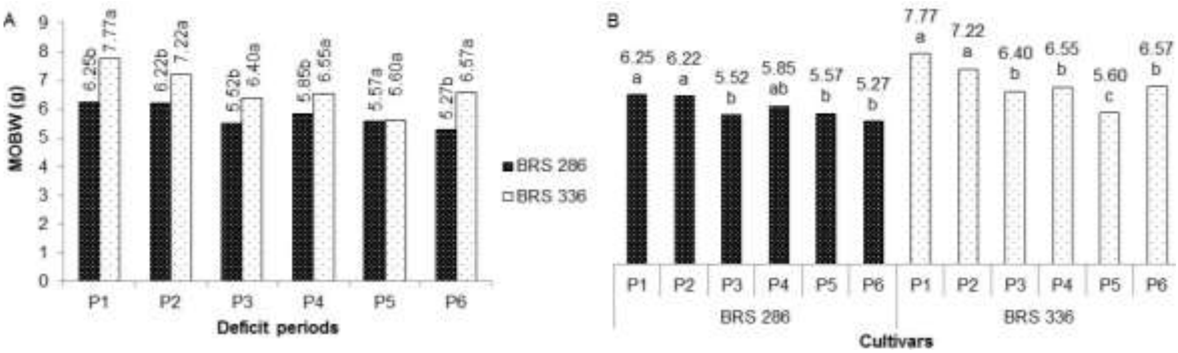

Figure 3. Development (A) of the cultivars in each deficit period and (B) deficit periods in each cultivar for mean open boll weight of two upland cotton cultivars under different water deficit strategies in the phenological phases. Pombal county, Paraíba state, Brazil, 2015. Same letters in the factors (A and B) indicate no significant difference among means (Tukey, p<0.05).

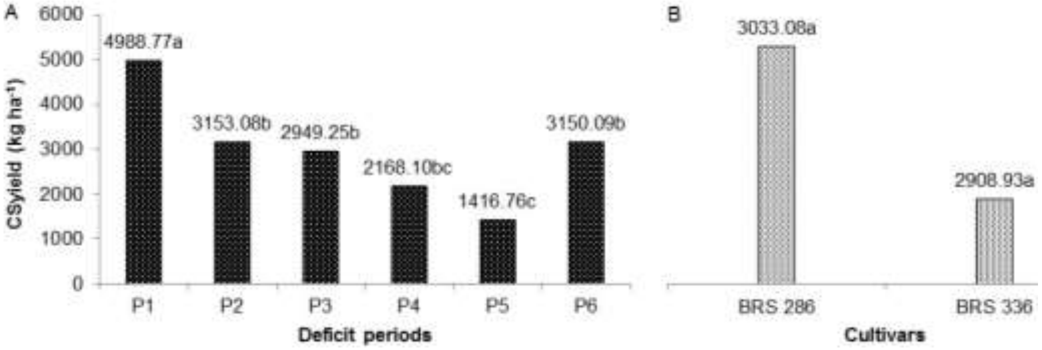

Figure 4. Mean cotton seed yield of two upland cotton cultivars under different water deficit strategies in the phenological phases (A. Deficit periods; B. Cultivars). Pombal county, Paraíba state, Brazil. 2015. Same letters in the Factors (A and B) indicate no significant difference among means (Tukey, p<0.05).

the water deficit on the lint technology and phenology of cotton CNPA 6H, and found similar results as this study when reporting a decrease in the MOBW per plant subjected to water stress.

The deficit periods affected CSyield which decreased when the plants had no irrigation in different phenological stages, that is in the stages of P2, P3, P4, P5 and P6, with mean reductions of 36.80, 40.89, 56.55, 71.61 and 36.86%, respectively, in relation to P1. The phenological stages P3, P4 (floration) and P5 (fruiting) were the less tolerant to water deficit as shown in Figure 4A. Such results were similar to Zonta et al. (2015) and Zonta et al. (2017) who stated that the deficit in cotton irrigation provided decreased CSyield, as a consequence of the sharp shedding of flowers and young bolls, which is reflected in crop yield and also to Onder et al. (2009) who showed that deficit irrigation causes a decrease in yield and yield components, as observed in this study.

Regarding the cultivars evaluated, BRS 286 and BRS 336 showed similar cotton yields (3,033.08 and 2,908.93 kg ha^{-1}, respectively) as shown in Figure 4B. Almeida et al. (2017), evaluating the production of upland cotton cultivars under water deficit, found similar results in terms of yield. These data also was similar to results obtained by Jalota et al. (2006) and Almeida et al. (2017) who stated that the stage of formation of open bolls (P5) is less tolerant to water stress and that water deficit promoted the fall of flower buds, flower abortion and/or shedding of bolls and open bolls, resulting in lower yield. Zonta et al. (2017), in turn, stated that when water deficit is applied in these stages (formation of flower and boll), the plant has a decreased formation and a marked shedding of reproductive structures (flowers and young bolls), which compromises yield, thus corroborating the results obtained in this study.

Sousa Júnior et al. (2005), Cordão Sobrinho et al. (2007) and Mendez-Natera et al. (2007) have reported that low levels of soil water caused a decrease in CSyield. In addition, the same authors have verified that water deficit reduces flowering and the retention of bolls

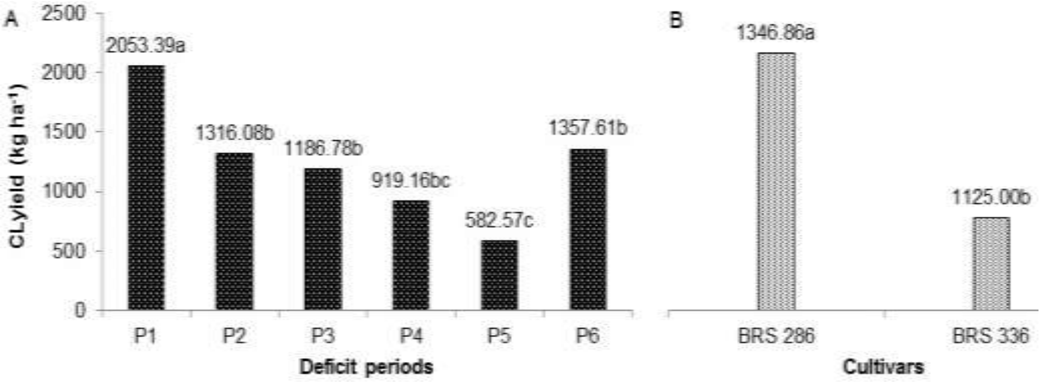

Figure 5. Mean cotton lint yield of two upland cotton cultivars under different water deficit strategies in the phenological phases (A. Deficit periods; B. Cultivars). Pombal county, Paraíba state, Brazil. 2015. Same letters in the Factors (A and B) indicate no significant difference among means (Tukey, $p<0.05$).

and causes the inadequate formation of the different parts of the plant such as stems, leaves and bolls, thus causing a decrease in yield. Adequate water availability provides increased yield. On the other hand, water deficit decreases yield (Nunes Filho et al., 1998; Cordão Sobrinho et al., 2007).

The behavior of cotton CLyield was similar to CSyield and it decreased when the plants had no irrigation in different phenological stages, that is in the stages of P2, P3, P4), P5 and P6, with mean decreases of 36.76, 37.45, 55.92, 73.46 and 35.90%, respectively, in relation to P1. The phenological stages of P3, P4 (floration) and P5 (fruiting) were the less tolerant to water deficit as seen in Figure 5A.

Cultivar BRS 286 showed higher CLyield because of its variety characteristics of higher fiber percentage in relation to BRS 336 (1,346.86 and 1,125.00 kg ha^{-1}, respectively) as presented in Figure 5B. Except for treatment P1 (without water deficit), mean values of CLyield were below the variety standard in all other deficit treatments as presented in Figure 5B, which is 1,995 kg ha^{-1} for cultivar BRS 286 and 1,527 kg ha^{-1} for cultivar BRS 336, according to Silva Filho et al. (2008) and Morello et al. (2011), respectively. CLyield was influenced by CSyield, and by F of the cultivars. Finally, the cultivars evaluated presented lower CLyield than the national average, which was 1,473.2 kg ha^{-1} in the 2016/17 season (Conab, 2017). The results presented was similar to Wen et al. (2013), who found decreases in CLyield when testing several cotton cultivars subjected to water deficit irrigation.

In general, the treatments with water deficit in the stages of P2 and P3 as shown in Figures 4 and 5 were less affected since the plant had time to recover from water stress, as observed in the study of the gas exchange of these cultivars when CSyield and CLyield were little impaired. The water deficit applied in the P6 also did not seriously influence yields, as most bolls were already formed at that stage. This comment was similar to Zonta et al. (2017) who stated that irrigation with controlled water deficit can be used in the cotton crop, with smaller irrigation depths in these stages (P2, P3 and P6), when the cotton is more tolerant to drought, which would increase the efficiency in the use of irrigation water.

Furthermore, Guinn and Mauney (1984) stated in their research that (severe) water restriction reduces cotton yield because of the decrease in the NOB per area, given the decrease in flowering and the shedding of young bolls. Other authors such as Pettigrew (2004) and Wen et al. (2013) also pointed out that water limitation in cotton causes the shedding of bolls and consequently lower yield. Loka and Oosterhuis (2012) stated that the reproductive stage is the less tolerant to water stress in the cotton crop, while Kock et al. (1990), Plaut et al. (1992) and Radin et al. (1992) stated in their works that the filling stage of the bolls is the less tolerant to water stress, which is similar to the results found in this study.

Cotton when subjected to treatment P3 presented lower F than the other deficit treatments, but it did not differ statistically from P1 and P5, whereas when the plant was subjected to treatment P6 it presented a higher F, but it was statistically equal to P4 as presented in Figure 6A. Thus, differences can be observed in F in relation to the water deficit periods, although F was higher than 40% in all stages in which the cotton plants underwent either water restriction or not, which is similar to the values/results found by Basal et al. (2009), Onder et al. (2009) and Hussein et al. (2011), who stated that F is not affected by water deficit but by the hereditary characteristics of the cultivars. Cultivar BRS 286 presented a mean of 44.47% above the variety standard that is 39.5 to 41.0% (Silva Filho et al., 2008); cultivar BRS 336 presented a mean of 38.67% within the variety standard that is 38.0 to 39.5% (Morello et al., 2011). Cultivar BRS 286 had a higher F than BRS 336 as presented in Figure 6B.

The results mentioned above was similar to Zonta et al.

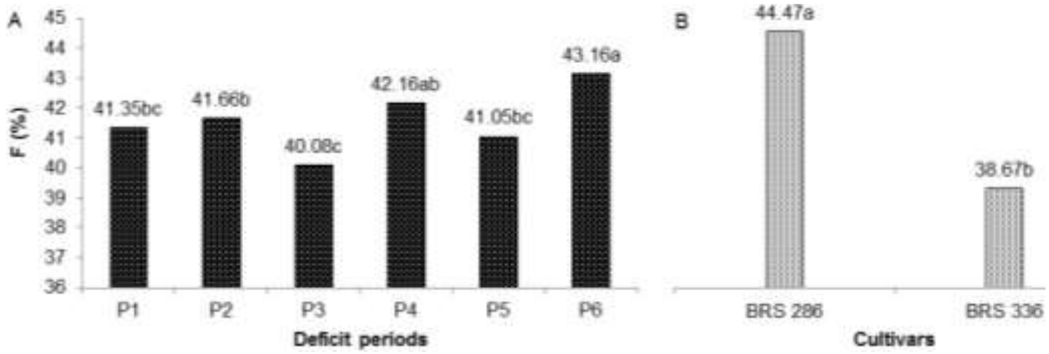

Figure 6. Means of fiber percentage of two upland cotton cultivars under different water deficit strategies in the phenological phases (A. Deficit periods; B. Cultivars). Pombal county, Paraíba state, Brazil. (2015). Same letters in the Factors (A and B) indicate no significant difference among means (Tukey, $p<0.05$).

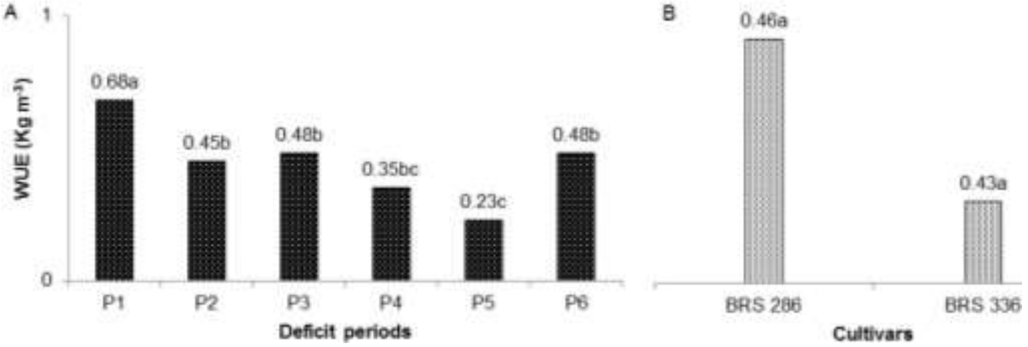

Figure 7. Means of water-use efficiency of two upland cotton cultivars under different water deficit strategies in the phenological phases (A. Deficit periods; B. Cultivars). Pombal county, Paraíba state, Brazil. (2015). Same letters in the Factors (A and B) indicate no significant difference among means (Tukey, $p<0.05$).

(2015) found when evaluating the effect of irrigation on CLyield and quality in which cultivar BRS 336 presented the lowest performance in CLyield, as well as the lowest performance in relation to F and CSyield. Opposite results were found by Almeida et al. (2017) when evaluating the production of upland cotton cultivars under water deficit, as they found different results in terms of F with treatments and cultivars that did not differ among themselves.

According to Zonta el al. (2017), when using irrigation with controlled water deficit, an important factor to be evaluated is the WUE of crops, especially in arid and semiarid regions, where water availability is limited. Cotton when subjected to treatment P1 presented higher mean WUE than the other deficit treatments, whereas when it was subjected to treatment P5 it presented the lowest absolute value, being statistically equal to only treatment P4 as presented in Figure 7A. Both cultivars presented the same WUE as shown in Figure 7B. The WUE decreased as the deficit periods were applied in the different phenological stages (from P1 to P6). As the applied volume was practically the same (low variation) from P2 to P6, what determined this variable was the yield, or rather, the effect of the deficit periods on yield, so that P2, P3 and P6 suffered the least effects. Compared to the results obtained by Embrapa Algodão (2006), whose overall WUE for cotton seed yield varies from 0.4 to 0.6 kg m^{-3}, all treatments are within this range, except for P4 and P5 (0.35 and 0.23 kg m^{-3}). The decrease in WUE in treatments with water deficit can be attributed to a decrease in the number of reproductive organs in relation to the vegetative ones, that is, a decrease in the harvest index. It should also be noted that in areas where water is a limiting factor, such as in the semiarid region, maximizing WUE is often more economically profitable for the producer than maximizing yield (Geerts and Raes, 2009). Zonta et al. (2017), working with 8 upland cotton cultivars subjected to water deficit at different stages of the crop, stated that the WUE behavior was very similar for all cultivars, varying between 0.39 and 0.84 kg m^{-3}.

According to last author, the worst results occurred in general for the treatment with water restriction in the stage of appearance of boll and flower and there was no statistical difference for the treatments with water restriction in the stages of initial growth and appearance of flower buds. In addition, most cultivars behaved very similarly when subjected to water deficit, regardless of the stage of the crop cycle, which corroborates the results found in this study.

Regarding the range of values, the WUE obtained can be considered high, except for treatment with water deficit in the stage P4 and P5, as Dagdalen et al. (2009), Singh et al. (2010) and Zonta et al. (2016) found values for WUE ranging from 0.4 to 0.8 kg m^{-3} in the well-irrigated treatments, that is, without water deficit. Zonta et al. (2017) demonstrated that irrigation with controlled water deficit can be an option to save water in cotton irrigation if it is carried out in the stages when the crop is more tolerant to water stress, which are the stages of initial growth, appearance of flower buds and appearance of open bolls.

Cultivar BRS 336 showed lower performance in the NOBP (7.63), CLyield (1,114.17 kg ha^{-1}) and F (38.19%), but it was better in MOBW (6.68 g); both cultivars were similar in performance in CSyield and in WUE.

In general, virtually for all variables studied, a decrease was observed when water deficit was applied in the periods of appearance of flower and boll. Corroborating this research, Bauer et al. (2012) stated that the problem of water deficit at the beginning of flowering is that the crop is acclimated to vegetative growth, which has no restrictions, as the plant is in optimal water conditions. According to Oosterhuis and Wullschleger (1987), the sudden water stress in a previously non-stressed plant can cause severe damage to plants. Brito et al. (2011) stated in their work that the reproductive stages coincide with the stage of increased water demand of the crop, which varies from 2.5 to 6 mm day^{-1}, thus, water deficit in these stages has more severe consequences as stated by Bauer et al. (2012). According to Yeates (2014), bolls are less affected by water deficit and will maintain growth after the leaves and internodes have stopped growing. This is because water is supplied to the bolls by the phloem and not by the xylem; therefore, they do not depend on the water potential gradient between the plant and the soil or atmosphere (Zonta et al. 2017).

Furthermore, according to Yeates (2014), abortion of fruit structures can occur up to 14 days after anthesis (<2 cm in diameter), when thickening of the cell wall between the fruit and the stem, prevents the formation of an abscission layer. Guinn (1982) presented another interpretation that large flower buds and flowers are more tolerant to shedding under water stress than young bolls, which corroborates the results obtained in this study.

Conclusions

Cultivars studied were more tolerant to water deficit in the stages of initial growth (P2), flower bud (P3) and open boll (P6). Water deficit during the flowers and bolls stages in upland cotton cultivars was the most detrimental to production components. Between cultivars tested, their behavior was similar only in cotton seed yield and water-use efficiency being BRS 286 higher than BRS 336 in the other analyzed variables, except for mean open boll weight.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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