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Full Length Research Paper

The effect of swine raising wastewater in the development of millet (*Pennisetum glaucum* L.), soil and leachate

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The experiment was conducted in a protected cultivation in the Marechal Cândido Rondon, PR, Brazil. The experimental design was constituted of randomized blocks with six piggery wastewater doses (0, 25, 50, 100, 200 and 400 m³ ha⁻¹) in four repetitions. The variables evaluated were: number of tillers, plant height, dry matter, leaf area, leaf number, potassium content, phosphorus content and crude protein in the culture, leachate and soil analysis. The number of tillers increased linearly with the addition of SRW doses. At the beginning of the development of millet culture, swine raising wastewater (SRW) application causes a decrease in plant height; however, over the course of time, this management increases those values. There was an increase in the number of leaves at the beginning, and leaf area at the end of the development of millet crop. The SRW doses applied did not cause increase in phosphorus and potassium contents in plants, raising only the crude protein. The dry mass is highly influenced by the increase in SRW doses, with their highest levels in a dose of 319.75 m³ ha⁻¹. The SRW doses cause reduction in soil pH and its constituents are not leached.

Key words: Fertilization, swine dejects, nutrient leaching.

INTRODUCTION

In the southern region of Brazil, pig farming is one of the most important activities, and it represents almost 50% of all national production (SEAB / DERAL, 2013). As a result of the increased production, a large volume of

waste is generated. Given its pollution potential, these wastes require specific treatments established by environmental protection laws that, in some situations, and given the inappropriate training of producers for the

management of such wastes, they are simply treated as pollutants (Cabral et al., 2011).

The use of swine raising wastewater (SRW) will be effective as a biofertilizer if performed properly, due to the fact that it contains macro and micro nutrients such as nitrogen, phosphorus (Hountin et al., 2000; Ceretta et al., 2010), potassium, calcium, sodium, magnesium (Queiroz et al., 2004; Ceretta et al., 2005), iron, zinc, copper (Girotto et al., 2006; Mattias et al., 2010) and others that can contribute to reduce the use of chemical fertilizers on crops, easing production costs, and thus, increasing the income of rural properties (Berwanger, 2006; Vielmo, 2008; Dal Bosco et al., 2008).

Pearl millet (*Pennisetum glaucum* (L.) R. Brown) is a grass of tropical origin, annual summer, easy deployment and management, which stands out for its adaptation to a wide range of environments and to different conditions of climate and soil, being characterized by its precocity, its high potential of production and its nutritional quality (Tabosa et al., 1999).

Among the species used as cover crops and green manure, millet (*P. glaucum* (L.) R. Brown), is characterized by the production of straw in large quantities and with characteristics of greater persistence of the soil (Silva et al., 2010), for its high capacity of soil nutrient extraction, with large advantages of recycling, especially N and K, reducing leaching risks (Leite et al., 2010).

The composition of the SRW varies according to the production system used in each farm, by setting the degree of dilution of waste and its physico-chemical characterization (Castamann, 2005). Thus, to meet the nutritional needs of a particular culture, there is a concern about the possibility of contamination of groundwater by the movement of ions (Melo et al., 2006). Another problem is the possibility of soil and water contamination due to the high concentration of heavy metals, such as Zn and Cu, which the pig slurry has (Gräber et al., 2005; Mattias, 2006).

According to Prior (2008), inadequate application without a previous assessment of possible impacts that may be generated by SRW, is one of the problems found in many properties in the western region of Paraná - which concentrates 40% of swine raising of the State. This happens due to the lack of technology enhanced to use this material, and it may possibly contaminate groundwater, rivers and soils.

The above work has been prepared on the assumption that the application of high doses of swine raising wastewater contributes in the development of millet, but at the same time, can cause contamination of soil and groundwater. In this context, this study was aimed to evaluate possible effects of the application of SRW rates, for the concentrations of macro and micronutrients in the soil, potassium, calcium, magnesium, manganese, iron, copper and zinc in the leachate, and growth parameters in culture millet, sown in Polyvinyl chloride (PVC) pipes.

MATERIALS AND METHODS

The experiment was conducted in a protected cultivation in the Horticulture Station and Protected Cultivation "Prof. Dr. Mario César Lopes to the Experimental Stations Center of Universidade Estadual do Oeste do Paraná - UNIOESTE, Campus Marechal Cândido Rondon, PR (coordinates 54° 22'W longitude and 24° 46'S latitude and an altitude of 420 m). The climate, according to Köppen (Critchfield, 1960) is the subtropical humid mesothermal Cfa dry winter, with rainfall well distributed throughout the year and hot summers. The average temperature of the coldest quarter varies between 17 and 18°C and in the hotter quarter between 28 and 29°C (IAPAR, 2007).

The treatments applied to the plots consisted of six SRW application rates (0, 25, 50, 100, 200 and 400 m³ ha⁻¹), and 24 soil columns made of PVC pipe cross-section of 0.20 m in diameter and 0.70 m long were used, with a capacity of 19 L of soil, considering each column as an experimental unit.

At the bottom of the tube, a 20 mesh plastic screen was attached. A 5 cm layer of crushed stone No. 1 was used to help in the leachate filtering. The tubes were coupled in 18 L plastic pots (with closed bottom) to store the leachate and the spaces between the tube and the pots were sealed with a plastic foil, to avoid the mixture of the irrigation water along with the leachate. The sections were filled with soil, close to the surface.

The soil used in the experiment classified as eutrophyc red latosol (Oxisol) was collected from agricultural land at 0 to 20 cm and after being dried in shade and sieved on a 0.4 mm sieve, we carried out sampling for chemical caracterization (EMBRAPA, 2009), whose analysis showed the following characteristics: pH 4.44; Mo (mg dm⁻³): 20.51; P (cmol$_c$ dm⁻³): 16.91; H + Al (cmol$_c$ dm⁻³): 4.85; Al +3 (cmol$_c$ dm⁻³): 0.2; K (cmol$_c$ dm⁻³): 0.46; Ca (cmol$_c$ dm⁻³): 5.26; Mg (cmol$_c$ dm⁻³): 1.15; SB: 6.87% and V: 58.67. Liming was done according to the methodology of Gianello et al. (1995), getting set the amount of 1.77 t ha⁻¹ dolomitic limestone with PRNT 75% to 415 g in all the work for soil acidity correction.

The millet sowing (cv. BRS 1501) was manually held on October 26, 2012 leaving only one plant per section. We added about 30 g of straw (composed of Tifton grass hay) around the plants to protect the soil, by preventing the surface compaction caused by irrigation. The irrigation was manual and daily, with the help of sprinklers in accordance with the needs of the crop during its development.

The SRW was collected in a rural property located in the district of Novo Três Passos, in the city of Marechal Cândido Rondon – PR, in a pig farm in the finishing phase, which had already gone through the anaerobic process in an indian digester. Two samples of the residue were collected. One sample was sent to the laboratory for evaluation of total N (Methods of Analyses of the Association of Official Analytical Chemistist). The other sample was stored in a plastic bottle and frozen for a period of 15 days. After thawing, the sample was subjected to perchloric nitro digestion, according to the methodology described by Lana et al. (2010), for analysis of other chemical elements. The analysis showed the following composition: N (mg L⁻¹): 2240; P (g kg⁻¹): 0.4; K: (g kg⁻¹): 9.79; Ca (g kg⁻¹): 0.89; Mg (g kg⁻¹): 0.5; Cu (mg kg⁻¹): 36; Fe (mg kg⁻¹): 77.25; Mn (mg kg⁻¹): 12.75; Zn (mg kg⁻¹): 26.5.

The application of SRW was held on November 15, 2012, at the beginning of the vegetative cycle, before the culture starts its tillering (20 days after sowing - DAS). SRW was manually applied with the aid of a pipette, which was also used to dose the amount applied per plot, taking great care of the residue to be deposited uniformly over the entire area of the PVC pipe. The prescribed doses were based on an average of swine manure applied per hectare with manure spreader, about 150 m³ ha⁻¹, commonly used by producers in the region as a means of material waste and grazing fertilization. The doses were applied following the randomized complete block design with four replications.

Figure 1. (a) Surface plot representing of number of tillers under the effect on doses of swine wastewater, from 20 to 48 days after sowing. (b) Surface plot representing of number of tillers under the effect on doses of swine wastewater, from 48 to 76 days after sowing.

During the experiment at 20, 27, 34, 41, 48, 55, 62, 69 and 76 days after sowing (DAS), the tiller number and plant height were evaluated. In the count of the tillers, all those which started tillering process were considered. For the plant height measurement, a measuring tape graduated in cm was used, and the distance between the ground surface and the maximum height reached by the leaves were measured, considering the point at which they are bent.

At 48 and 76 DAS, an evaluation was made of the dry mass of stem + sheath, dry mass of leaves, leaf area, leaf number, nitrogen content, potassium and phosphorus in plants, concentrations of potassium, calcium, magnesium, manganese, iron, copper and zinc in the leachate, dry weight of roots and waste, as well as macro and micronutrient concentrations in the soil. To obtain the dry weight values, the plants were cut at a height of 0.20 m above the ground, separated into leaves and stems plus sheath, and packaged in paper bags. Later, they were taken to a forced air circulation oven at 65°C for 72 h to determine the dry weight, which, when are added, represent the total dry matter according to the methodology described by Benincasa (2003).

To determine the dry mass of the root system, the pots were broken and the roots washed under running water to remove the excess of soil with subsequent drying in a forced air circulation oven. For leaf area determination, the authors used the destructive method of area known in cm^2, considering the dry mass of leaves (DML) and dry mass of the known area of sample (sample DM) according to the methodology described by Silva and Queiroz (2002).

After drying the fraction of the aerial part of the plant, the stems + sheath and the leaves, as well as the root system and the residual of the stems, they were ground separately in Wille type mill and weighed samples of 0.20 grams for further realization of sulfuric digestion. The digestion product ammonium sulfate [(NH4) 2SO4] was treated with excess of strong base (sodium hydroxide - NaOH - 10 mol L^{-1}) and subjected to distillation. With the values of the volume spent in the titration, the nitrogen (N) calculations were done in g kg^{-1} and the protein ones by multiplying the value of N by 6.25. After dilution of the digested material, the reading was carried out in a flame photometer previously calibrated and using the equation, the concentrations of potassium (K) in g kg^{-1} were obtained. By reading absorbance performed in a spectrophotometer at 725 nm, the phosphorus concentration values in kg^{-1} g were

obtained, according to the methodology described by Lana et al. (2010).

At 76 DAS, the pots were broken, soil samples from each pot in depth 0 to 20 cm were packed in plastic bags, taken to the Physics Laboratory and Soil Classification from UNIOESTE, where they were transferred to paper bags and taken to the oven at 105°C until the obtainment of constant weight. Later they were sent to the Environmental Chemistry Laboratory and Instrumental from UNIOESTE and subjected to analysis for the determination of macro and micronutrients according to the methodologies described in Raij et al. (2001).

The leachate samples were stored in plastic bottles and frozen until the time of reading. Twenty-four hours prior to the readings, the samples were removed, thawed and sent to the Environmental Chemistry Laboratory and Instrumental from UNIOESTE, where readings were performed to determine the composition of its nutrients: K, Ca, Mg, Cu, Zn , Mn and Fe.

After being tabulated, the data were submitted to analysis of variance to verify its significance. When there was a significant effect, the average of the variables assessed over the time was studied by response surface with the aid of computer application GENES (Cruz, 2013). On the other hand, the averages of the variables assessed only at 48 and 76 DAS were studied by regression analysis, using the software Sisvar (Ferreira, 2011).

RESULTS AND DISCUSSION

The response surface generated by the analysis of the variable number of tillers, evaluated in the first period of 20 to 48 DAS (Figure 1a), in relation to the applied doses of SRW, was squaring in time and increasing linear over time regarding the doses. Furthermore, it was observed that the increase in doses slows the maximum production of tillers, showing that this variable takes more time to stabilize with higher doses. The equation found is from Z = -5.97967 + 0.36822x − 0.00365x² - 0.00768y + 0.0003xy with R² = 0.86, the value of X = 25.6; Y = -604.4 and the maximum point of 1.054698 tillers.
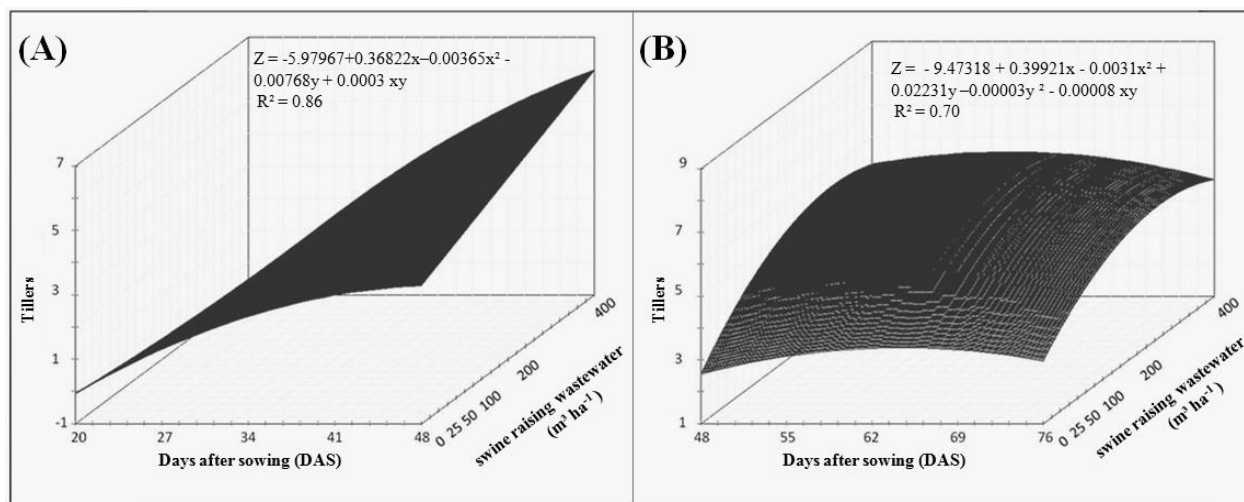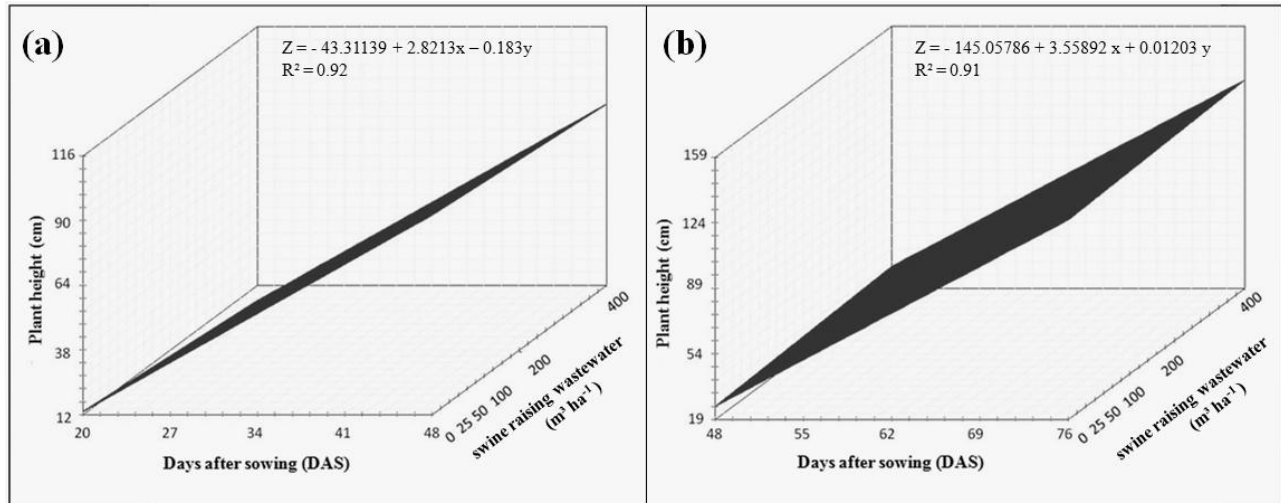
Figure 2. (a) Surface plot representing of plant height under the effect on doses of swine wastewater, from 20 to 48 days after sowing. (b) Surface plot representing of plant height under the effect on doses of swine wastewater, from 48 to 76 days after sowing.

In the second period (Figure 1 (b)), the effect was quadratic over the time and with an increase in the doses. The generated equation is $Z = -9.47318 + 0.39921x - 0.0031x^2 + 0.02231y - 0.00003y^2 - 0.00008xy$ with R^2 0.70. To 60.63 days after sowing and at a dose of 290.99 m³ h⁻¹, the maximum point found to the equation was 5.88 tillers.

The increase in tillering is associated with the higher availability of nutrients, which provides increased speed of formation of buds and initiation of the corresponding tillers. According to Freitas et al. (2005), appropriate fertilizer levels provide maximum tillering, while deficiencies increase as the number of dormant buds, reducing the number of tillers. Nevertheless, Mondardo et al. (2011) observed an increase in the number of tillers to the dose of 50 m³ h⁻¹, with subsequent reduction.

There was an increase in plant height in the first assessment period (Figure 2 (a)) increasing linearly over time. However, the increase of SRW doses caused a small decrease of this variable, the equation $Z = -43.31139 + 2.8213x - 0.183y$ with R^2 of 0.92. In the second period the behavior was also in a linear crescent way, despite the effect of the doses being small. The equation found for this variable was $Z = -145.05786 + 3.55892x\ 0.01203y$ with R^2 of 0.91. In both, the authors observed that the days after sowing had greater effects on plant growth than the doses applied. In other words, in every day that passed the plants increased 2.8213 and 3.55892 cm high respectively, and each m² of SRW applied per hectare increased 0.183 and 0.01203 cm high respectively.

Geraldo et al. (2000) when assessing the growth of four cultivars of millet (BN-2, IAPAR, HKP and Guerguera) observed that the average of plant height at 25, 30, 45 and 60 days after sowing was 6, 10, 56 and 170 cm,

respectively. On the other hand, Sobrinho et al. (2008), in his work which were evaluated fertilizer sources in millet cultivation in the semi-arid, found no differences in plant height when compared the fertilization sources (legume - 95.61 cm, NPK - 111.30 cm, cattle manure - 113.61 cm and goat manure - 132.47 cm) but only the legume was like the control that had an average height of 36.04 cm.

Regarding the leaf area, we observed that there was only a statistically significant difference of 5% of probability for the second cut (Figure 3 (a)), whereas in the number of leaves (Figure 3 (b)) there was difference in the first cut, both in a linear crescent way. The leaf area depends intimately on soil fertility, climatic conditions, the spacing and the genetic material efficiency in using the available nutrients. As photosynthesis depends on the leaf area, the crop yield will be greater the faster the plant achieves the maximum leaf area and the longer this variable remains active (Pereira and Machado, 1987).

Tomazella (2005) attributes that the increase in the leaf area is due to the larger number of photosynthetically active leaves, brought about by the expansion of leaf length. The number of leaves is a fairly stable genotypic characteristic; however, although it is genetically determined, it is also affected by environmental and handling factors (Mondardo, 2010). According to Costa et al. (2005), being the leaf the carbohydrate production center that will supply the vegetative and reproductive organs, its sanity and also its number are the essential factors to ensure the effective yield of the crop.

Gonçalves and Quadros (2003) had 4.2 leaves per tiller when evaluating a millet pasture under cattle grazing. On the other hand, Mondardo et al. (2011) found 6.32 leaves per tiller at a dose of 75 m³ ha⁻¹ of swine manure. Through analysis of variance, there was no significant
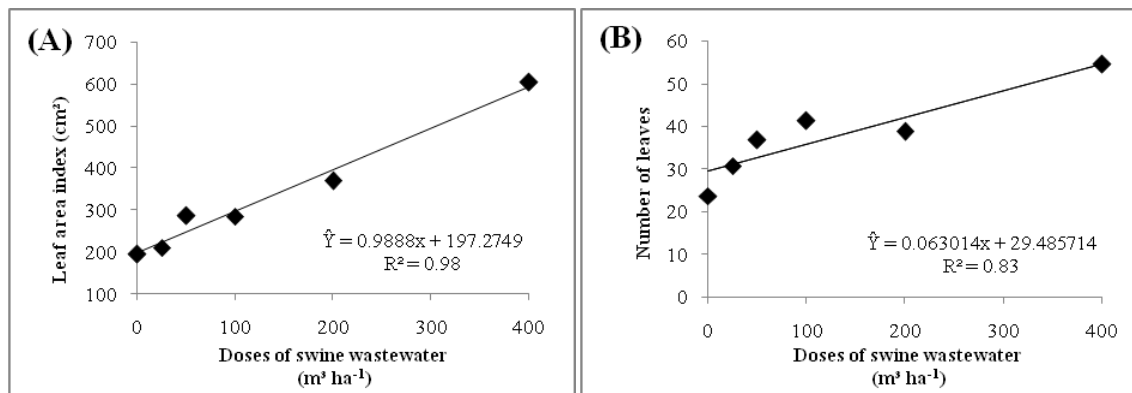
Figure 3. (A) Leaf area of second cut –76 DAS, (B) Number of leaves of first cut – 48 DAS.
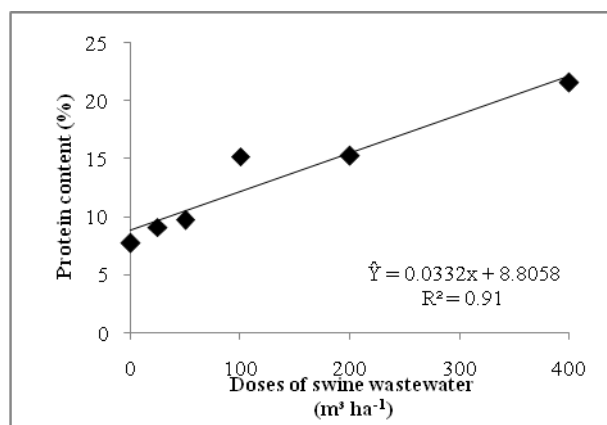
Figure 4. Crude protein content (%) of millet culture under the effect on doses of swine wastewater.

statistical difference at 5% probability for the potassium and phosphorus contents in the fractions of the evaluated plants, as to the content of protein in the plant; there were significant differences in the levels found in the first cut leaves.

This result is related to the nitrogen content that the culture absorbed during its development, since this nutrient is important for the development of the culture. According to EMBRAPA (2003), with the purpose of holding the fodder, silage or grazing, it is recommended to apply between 20 and 30 kg of N ha^{-1} at sowing and 60 to 80 kg N ha^{-1} in cover at the beginning of tillering. If planting is carried out in spring / summer in areas that have not suffered any previous fertilization, the soil must be corrected as if to planting a forage of average demand, with the nitrogen being used at the base, 50-100 kg ha^{-1} (EMBRAPA, 2003).

Through the study by regression analysis, we observed an increase of protein in the leaves in the first cut (48 DAS) in a linear crescent way, as the applied SRW doses were increased (Figure 4). Amaral et al. (2008) when studying three varieties of millet (BRS1501, BN 1 and

Common) at 70 days of growth, found an average crude protein content (9.86%) similar to those obtained with the lowest doses studied by Mondardo et al. (2011), that in assessing the behavior of millet under doses of swine manure culture, observed quadratic effect on crude protein content, being the lowest content (8.77%) obtained with doses of 13 m³ ha^{-1}, from which the content rose to 13.82%, with a maximum dose of manure (115 m³ ha^{-1}) applied. Heringer and Moojen (2002) also observed an increase in crude protein in response to nitrogen application in millet. On the other hand, Kollet et al. (2006) found 15.42% values of protein for millet with 42 days of age. Mondardo et al. (2011) observed an increase in crude protein of oat in response to application of pig slurry doses up to 50 m³ ha^{-1}.

Guideli et al. (2000) when evaluating production and quality of millet fertilized with nitrogen, noted that crude protein showed differences between fractions, being greater in the leaf (22% on average) in relation to the stem (14% on average). Similar behavior was observed by Aita (1995), in millet common cultivar fertilized with300 kg ha^{-1} N, whose average protein content in the leaf
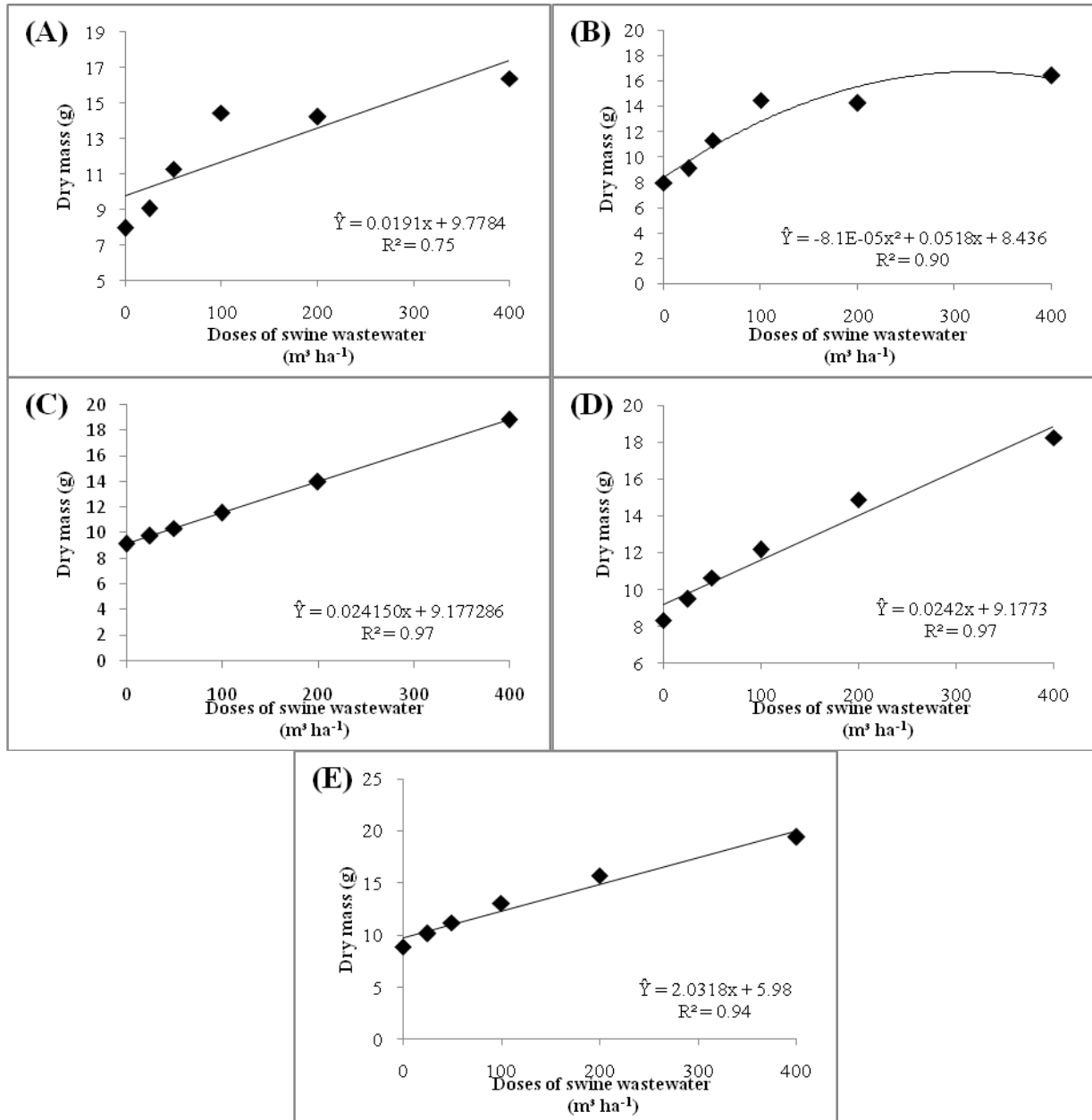
Figure 5. (A) Leaf dry mass of the first cut, (B) Leaf dry mass of the second cut, (C) Culm and sheath dry mass of the second cut, (D) Residual dry mass, (E) Total dry mass of millet culture under the effect on doses of swine wastewater.

and stem was 11.3 and 7.4%, respectively. However, in this paper, the protein content only found in the first cut leaves increased 13.83% from the higher dose applied (400 m³ ha⁻¹), in relation to the low treatment (0 m³ ha⁻¹). Since this was a linear effect, it is believed that plants fertilized with doses above 400 m³ ha⁻¹ of SRW in the studied conditions, have higher crude protein contents. When evaluating the leaves dry mass, stem + sheath, roots, residual, and total dry matter of the culture, by analysis of variance, we observed a significant statistic difference at 5% of probability for dry mass of leaves,

both for the first and for the second cutting, dry matter of the stem + second cut sheath, for residual dry matter mass and, consequently, for total dry matter mass. Through a study using regression analysis (Figure 5), it can be seen that there is an increase in the dry matter mass of the first cut leaves, stem dry matter plus sheath of the second cut, residual dry matter and dry matter mass of the total plant, linearly. As for the second cut, the increase in dry matter mass in the leaves occurred in increasing quadratic response.

The dry matter mass of the second cut sheets had its
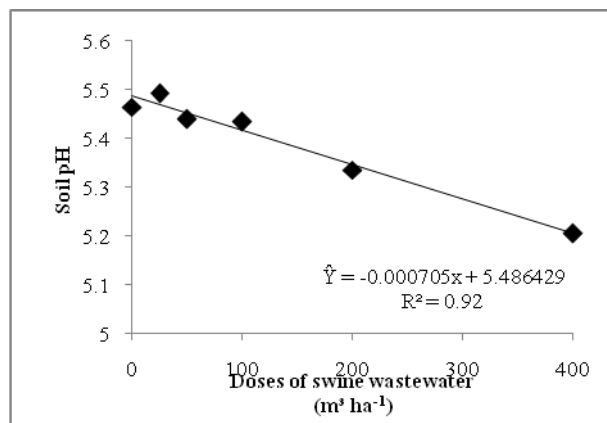
Figure 6. Soil pH after the cultivation of millet under the effect on doses of swine wastewater.

higher yield with the dose of 319.75 m³ ha^{-1} of SRW, with dry matter mass of 16.72 g per plant. Mondardo et al. (2011) also found a quadratic effect of the application of liquid swine manure on the dry matter mass production of leaves, stem and root system (p > 0.05). The dry matter mass of stems decreased to the dose of 43 m³ ha^{-1}, while the dry matter mass of the leaves and root system rose to the doses of 75 and 50 m³ ha^{-1}, respectively. The increase in stems' dry matter mass is a result reported in the literature for the application of nitrogen fertilization (Gomide, 1997; Patês et al., 2007), and this characteristic is essential in the development of tiller and consequently on grazing.

Due to the fact that pig waste has a high concentration of N in its composition, about 2.24 kg of N m^{3}, the dose of 400 m^{3} h^{-1} achieved limits of 890 kg ha^{-1}, on its higher dose, much higher than the recommended, which may have favored this improvement in the development. Likewise, Bellon et al. (2009) found quadratic behavior of the variables in response to doses of swine manure. The minimum dry matter mass production of the stems was obtained with the dose of 45.5 m^{3} ha^{-1} of swine manure. The maximum dry matter mass production of leaves, shoots and roots were obtained with doses of 67.9; 65.6 and 56 m^{3} ha^{-1}, respectively, equivalent to N doses 89.3; 86.3 and 73.6 kg ha^{-1}.

Buso et al. (2012) when evaluating the dry matter mass production of millet cultivars subjected to various levels of nitrogen fertilization, observed quadratic effect in relation to the application of N and dry matter mass production. On the other hand, Nobrega (2010) and Silva (2010) observed linear effect according to an increase of N doses with the highest production achieved with 80 and 160 kg ha^{-1} of N, respectively, in work conducted with millet.

There was no significant difference between most of the nutrients and soil characteristics evaluated in terms of the SRW doses in millet cultivation. Only the pH was

influenced by this source of variation. From the data that consisted significant effect of SRW doses, they were studied by means of regression analysis. There was a linear decrease in the soil pH, decreasing with the increase of applied dose (Figure 6).

In soil with a pH near neutrality, it is possible to find a decrease in pH with the use of the manure (Lourenzi, 2010). This has been observed in the United States by Adeli et al. (2008), who used pig slurry in a soil with an initial pH of 6.9 and, after applying the manure for 15 years, the soil pH decreased to 5.9 in the layer of 0 to 15 cm. According to Lopes (1989) and Guerra et al. (1999), the decrease of the soil pH values over time is due to leaching of bases along the soil profile. Medalie et al. (1994) emphasize that the greater the amount of nitrogen fertilizer, the greater the acidity of the soil due to the release of H + ion.

Conversely, Assmann et al. (2007) working on an eutrophyc red latosol (Oxisol) with an initial pH of 4.52, in the layer of 0 to 20 cm, and applying twice the dose of 80 m^{3} ha^{-1} of swine manure, in a period of 156 days observed that the pH rose to 4.79. For Hue and Licudine (1999), the addition of organic residues in acid soils can cause increased pH values in soil water by the adsorption of H + ions in the decaying crop residues, which are part of the non humic fraction of soil organic matter.

Chantigny et al. (2004) reported that increments in soil pH values can be due to the alkaline characteristic of swine manure and also by the dissociation of the manure derived carbonates.

Cassol et al. (2012), when assessing the availability of macronutrients and corn yield fertilized with swine manure applied superficially in dystroferric red latosol, whose pH in the layer 0 to 20 cm was 6.1, cultivated with oats-corn succession in direct planting system in annual doses of up to 200 m^{3} ha^{-1}, for nine years, realized that no changes were found in the soil pH. These results agree with assessments of other works, which also found

no effect of this kind of animal waste in soil pH Ceretta et al. (2003), Scherer et al. (2010) and Cassol et al. (2011). According to Lourenzi (2010), there is a soil pH balance trend when it receives successive manure application, in a range of approximately 5.0 to 6.0.

The values resulting from the analysis of variance of K, Ca, Mg, Cu, Zn and Fe found in the leachate, collected in the two seasons respectively, were not significant. Prior (2008) when assessing the SRW effect in soil and corn, also did not find potassium in the leachate. However, Maggi et al. (2011), when evaluating the leaching of nutrients in soil cultivated with the application of SRW found concentrations of 84.94 mg L^{-1} K in the leachate. Maggi et al. (2011) also found higher Ca concentrations in the leachate as the increase in rates of SRW, and by regression analysis, the authors observed that this increase was in a linear way, since when comparing the leaching of this mineral, over the time the Ca contents leached were decreasing.

As the calcium in the soil has a stronger absorption when compared with Ammonium, Potassium and Magnesium, its leaching is not so intense and actually not enough to cause concern in terms of loss. This may explain the fact that this mineral does not have significant values when compared to the control. Nevertheless, authors with Furtini Neto et al. (2001) confirmed that the application of the organic waste in the soil increases the leaching of calcium due to the fact that SRW causes the increase of pH, promoting mineralization by increasing the release of CO_2 and consequently the leaching of Ca $(HCO_3)_2$ with water.

Although the absence of copper in the leachate was observed, especially when considered the treatments that received higher doses of SRW, the results corroborate Santos (2010), who also did not find the presence of this element in the leachate. Low Cu mobility in the soil was also commented on by Miyazawa et al. (1996), since Cu is a transition metal, has high affinity (high constant of stability) with organic compounds such as humic acid, fulvic acid, and organic acids soil.

Although pig manure has low concentrations of Cu and Zn, their application in excessive doses can result in accumulation of these elements in the soil, which can cause poisoning not only to plants, but also in other levels of the food chain (Scherer et al., 1996). Mancuso and Santos (2003), in general, also state that the heavy metals may be toxic to plants and animals. However, there are no case reports of chronic toxicity to plants and animals, due to the disposal of wastewater in the soil as a result of the low concentrations of these elements in the soil in wastewater. As for the infiltration and percolation, heavy metals are retained by the majority of soils, especially when high in organic matter and pH > 7.0. Another factor that contributes to the low mobility of heavy metals is the high clay content in the soil (Kabala and Singh, 2001).

The availability of Mn in the soil depends mainly on the pH, redox potential, the organic matter and the balance with other cations (Santos, 2010). When the pH of soil decreases the H + and Al^3 + Mn compete with the exchange sites, increasing the solubility of Mn in solution (Santos, 2010). Lamy (1983) suggests that although the amount of leached trace elements is less than 1% of the total added, there may be considerable increase when considering the sandy soils with low organic matter content and subject to heavy rains. This may explain the fact that Mn in the assessed leachate was not found, because clay was used as a substrate.

Conclusion

The number of tillers increases linearly with increments in SRW doses. The SRW application causes an increase in plant height from DAS 48 and 28 days after the first cut. The leaf area, dry matter mass of stem + sheath, residual dry matter mass and total dry matter mass, evaluated at 76 DAS and the number of sheets, crude protein content, dry matter mass of leaves assessed at 48 DAS showed linear crescent response to the increase in SRW doses.

The dry matter mass of leaves at 76 DAS had its highest yield with the dose of 319.75 m^3 ha^{-1} of SRW, with a dry matter mass of 16.72 g per plant. The K and P contents in the millet crop, K, Ca, Mg, Cu, Zn, Mn and Fe in the leachate were not affected by doses of SRW. Furthermore, in the soil before, only the pH had changed in relation to the applied doses, decreasing linearly. Finally, before the use of high doses of SRW causes changes in soil pH.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Do desiccation and storage of *Campomanesia adamantium* (Cambess.) O. Berg (Myrtaceae) seeds affect the formation and survival of seedlings?

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The objective of study was to verify if desiccation and storage of the seeds affect the morphoanatomy and histochemistry of the seedlings of *Campomanesia adamantium*. The fruits were processed and seeds were subjected to desiccation to 30, 20, 15, 10 and 5% water contents in laboratory conditions and subsequently subjected to the following storage conditions: In the laboratory (LAB) (25 ± 2°C, 35% RH), cold and dry chamber (CC) (16 ± 2°C, 40% RH), refrigerator (REF) (8 ± 2°C, 35% RH), and freezer (FZ) (-18 ± 1°C, 42% RH) for 0 (newly processed), 30, 60, 90, 120, 150, and 180 days. The evaluation of the seedling survival rate was performed for 42 days, calculated as the survival percentages for seedling shoot and primary root. Anatomical observations and histochemical tests were performed using fixed and non-fixed samples of the median region of xylopodium in normal and abnormal seedlings. In the morphoanatomy of normal seedlings, we observed cotyledons, hypocotyl, xylopodium, and well defined primary root and in abnormal seedlings, cotyledons, hypocotyl and xylopodium (with 20 and 15% water content), and hypocotyl and primary root (with 10% water content). The desiccation and storage of seeds affected the formation of seedlings by preventing the normal development of roots and shoots. The xylopodium of normal and abnormal seedlings showed positive reaction to starch and lipophilic substances. The presence of phenolic compounds and fructans were observed in parenchyma cells of the xylopodium in abnormal seedlings and absent in normal ones. The deleterious effects of desiccation in association with storage induce the production of phenolic compounds and fructans in abnormal *C. adamantium* seedlings.

Key words: Brazilian Savanna, xylopodium, water content, morphoanatomy, histochemistry.

INTRODUCTION

Seed storage is a safe and economical way to preserve genetic diversity of native plant species and represents a strategy to meet the continuous demand for seedlings for commercial purposes, reforestation, and recovery of

degraded areas (Costa, 2009). However, the success of seed storage depends on understanding the behavior of these seeds during the storage process, which enables the use of appropriate conditions to maintain their viability (Hong and Ellis, 1996). The most important feature in seeds destined for long-term storage is longevity, which includes the seed survival time (Hay et al., 2010).

The storage capacity is expanded to many species when the reduction in seed water content is associated with decreases in environmental temperature (Walters et al., 1998). However, some species do not tolerate sharp decrease in temperature such as freezing due to the damage caused by negative that cause the formation of ice crystals inside the tissues, and consequently, the loss of seed viability (Chin et al., 1989; Fonseca and Freire, 2003).

Campomanesia adamantium (Cambess.) O. Berg (Myrtaceae) is a native non-cultivated fruit tree, abundant in the Cerrado area of Mato Grosso do Sul, and sometimes present beyond Brazil, reaching areas in Uruguay, Argentina, and Paraguay (Arantes and Monteiro, 2002; Lorenzi et al., 2006). The leaves and fruits have anti-inflammatory, antidiarrheal, and antiseptic properties in urinary tract infections (Vieira et al., 2011). Fruits collected at different ripening stages showed potential for "*in natura*" use, in food industry and as flavoring agent in beverage industry due to high levels of acidity (1.2 g in citric acid) and ascorbic acid (234 mg 100 g^{-1} vitamin C), minerals (K -1304 mg kg^{-1}, Ca, P, and Mg from 165 to 175 mg kg^{-1} concentration and the microelements Fe -11.3 mg kg^{-1} and Al -15.9 mg kg^{-1}) dietary fibers, and monoterpene hydrocarbons (a-pinene (10.6%), limonene (10.1%) and the b-(z)-ocimene (9.2%) present in greater amounts in the essential oil of the fruits and which give them the citrus scent (Vallilo et al., 2006).

A large number of fruit trees and forest species have seeds that are sensitive to desiccation (Villela and Peres, 2004); however, the information gathered about the storage of seeds that belong to the genus *Campomanesia* is still contradictory. Although *C. adamantium* is classified as recalcitrant, intolerant to storage and desiccation over a silica gel at water content of less than 15% (Dresch et al., 2014), it is suggested that desiccation and storage of its seeds change the formation of the seedlings.

Thus, an important tool to determine the changes in seedlings resulting from desiccation and storage are the morphoanatomical and histochemical studies that contribute to the identification of substances, present in storage organs and which may be part of adaptive mechanisms to adverse conditions. Therefore, this work aimed to verify if desiccation and storage of the seeds affect the morphoanatomy and histochemistry of the seedlings of *C. adamantium*.

MATERIALS AND METHODS

C. adamantium fruits were collected at the end of December 2011,

from 30 tree samples in the Cerrado area (*sensu stricto*), in Ponta Porã- state of Mato Grosso do Sul (MS). After collection, the fruits were taken to the Laboratory of Plant Nutrition and Metabolism at the Federal University of Grande Dourados (UFGD) in Dourados-MS, where they were washed in running water and damaged fruits were discarded. Afterwards, the fruits were processed manually using sieves for separation of seeds. Then, the seeds were washed in running water and placed on Germitest® paper for 40 min at laboratory temperature (25 ± 2°C, 32% relative humidity - RH) to remove excess water (surface drying).

After, the seeds (fourteen thousand units) were dried under laboratory conditions on plastic trays and weighed every hour until they achieved the pre-established water content (30%, 20%, 15%, 10% and 5 ± 2°C) according to Sacandé et al. (2004) formula.

When the closest desired water content was achieved, a sample was homogenized, and divided into fractions packaged in clear plastic bags with a thickness of 0.20 mm (100 seeds) and subjected to the following storage conditions: Laboratory (LAB) (25 ± 2°C, 35% RH), cold and dry chamber (CC) (16 ± 2°C, 40% RH), refrigerator (REF) (8 ± 2°C, 35% RH), and freezer (FZ) (-18 ± 1°C, 42% RH). After 0 (newly processed), 30, 60, 90, 120, 150, and 180 days of storage, seeds were pre-humidified in 100% RH and at 25°C under continuous light for 24 h to avoid damage due to soaking, and the following, characteristics were determined to assess the physiological potential:

1. Water content: Determined at 105 ± 3°C for 24 h using the incubator method (Brasil, 2009), in three replicates each with 5 g of seed samples, and the results were expressed as percentage water content on a fresh weight basis.
2. Survival rate of shoot and primary root: A Germitest® paper roll was used for sowing four replicates, each of 25 seeds that were maintained in B.O.D. (Biochemical Oxygen Demand) incubator at 25°C under continuous light. Evaluations were performed for 42 days after sowing, calculating the percentages of survival of the shoot area and primary root of the seedlings and the results were expressed in percentage (%).

The design was a completely randomized factorial scheme (4 temperatures/environments × 7 storage periods). Differences in the temperature data designated as significant by analysis of variance were compared using Tukey's test and storage periods were adjusted by regression equations at 5% probability using the SISVAR software (Ferreira, 2011).

Morphoanatomical studies

Anatomical observations were made in the median region of the xylopodium of the *C. adamantium* seedlings. The cross sections obtained free hand were clarified with 20% sodium hypochlorite and, after being washed in 2% acetic alcohol and distilled water, were subjected to double staining using astra blue and safranin (Bukatsch, 1972) and mounted on glycerinated gelatin (Dop and Gautié, 1928).

Histochemical tests (qualitative)

Histochemical tests were performed using ten fixed and ten non-fixed samples of xylopodium of *C. adamantium* seedlings. The presence of lipophilic substances was visualized by using Sudan III (Sass, 1958), lugol for starch (Kraus and Arduin, 1997), and ferric chloride for phenolic compounds (Johansen, 1940). The slides were mounted using distilled water and observed posteriorly. For the fructans analysis, xylopodium samples were treated with sulfuric acid, and subsequently viewed under polarized light (Johansen, 1940).

The morphoanatomical results were analyzed and illustrated by
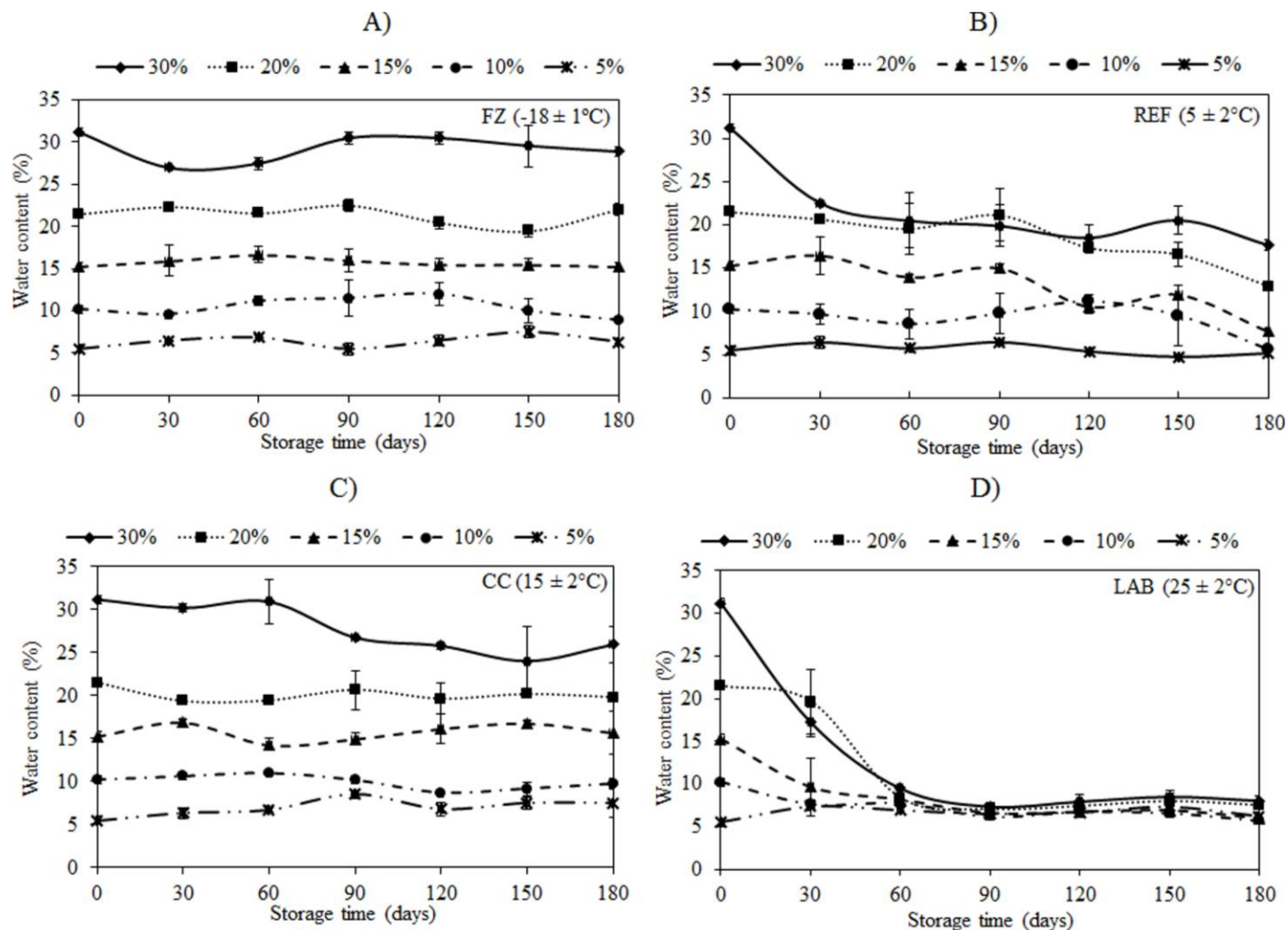
Figure 1. Water content (%) of *C. adamantium* seeds packed with different water contents (30, 20, 15, 10 and 5%), environmental conditions LAB - laboratory (A), CC - cold and dry chamber (B), REF- refrigerator (C), and FZ - freezer (D), and storage times (0, 30, 60, 90, 120, 150 and 180 days).

means of photographic equipment Sony Cyber-shot (Sony Electronics Inc., Japan) mounted on microscope Nikon Eclipse E 200 (Nikon Co., Tokyo, Japan). In all cases, scales were added according to the optical conditions used. The data for water content were presented as the average results and standard deviation.

RESULTS

The temperature of cold and dry chamber (16 ± 2°C), refrigerator (8 ± 2°C) and freezer (-18 ± 1°C) provided small variations in the water content levels during storage (Figure 1a to c). The seeds stored in laboratory temperature (25 ± 2°C) showed reductions in water levels over time, and these values were more pronounced in seeds with high water content (30 and 20%), so that at the end of 180 days showed water content of 8.0 and 7.5%, respectively (Figure 1d).

The survival rate of shoot and primary root was

influenced by desiccation, environmental conditions, and seed storage time (Figures 2 and 3). Initially, the survival rate of shoot and primary root primary root was not affected by the environmental conditions (temperatures and storage times).

The survival of the shoot of the seedlings with reduced storage time in all water contents, the levels of 30 in 15% survival was greatest when seeds were stored in cold and dry chamber (Figure 2a to c). When the seeds were stored with water content below 15% survival percentage was close to zero from the 30 days of storage (Figure 2d and e). The seeds stored for up to 60 days in cold storage and water content of 15% showed high survival percentage of the shoot (over 50%).

For the survival of the primary root, the results were similar to the shoot, however, the seeds stored in cold and dry chamber with 15% water content the survival was less than 50% at 30 days (Figure 3a to e).
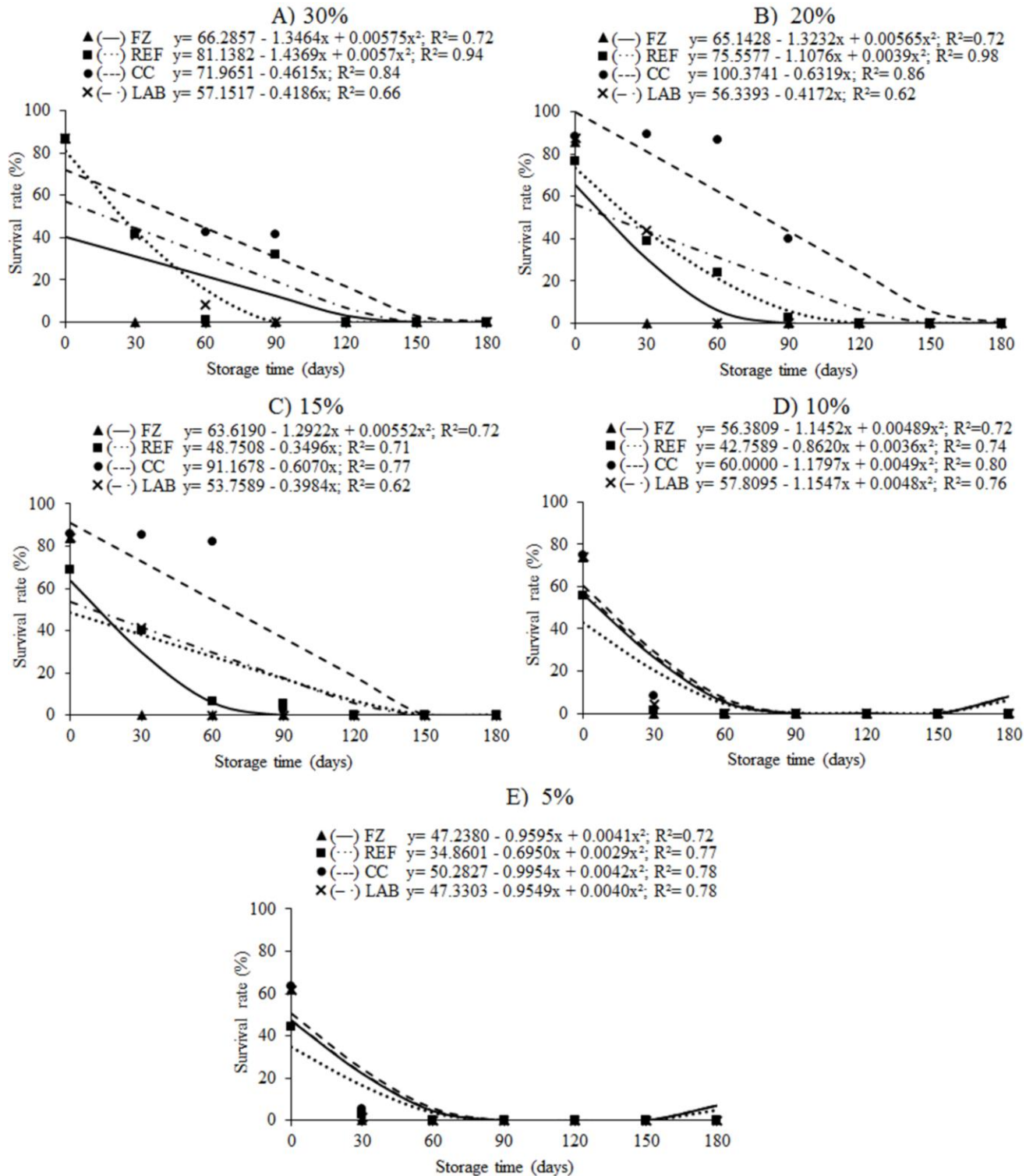
Figure 2. Survival rate of shoot of *C. adamantium* seedlings stored at different water contents of 30 (A), 20 (B), 15 (C), 10 (D) and 5% (E), environmental conditions (LAB - laboratory (25°C), CC - cold and dry chamber (16°C), REF- refrigerator (8°C), and FZ - freezer (-18°C), and storage times (0, 30, 60, 90, 120, 150 and 180 days).

Starting at 30 days of storage, we found that the seeds stored under freezer conditions showed no germination and consequently no formation of seedlings over the 180 days of storage (Figures 2 and 3).
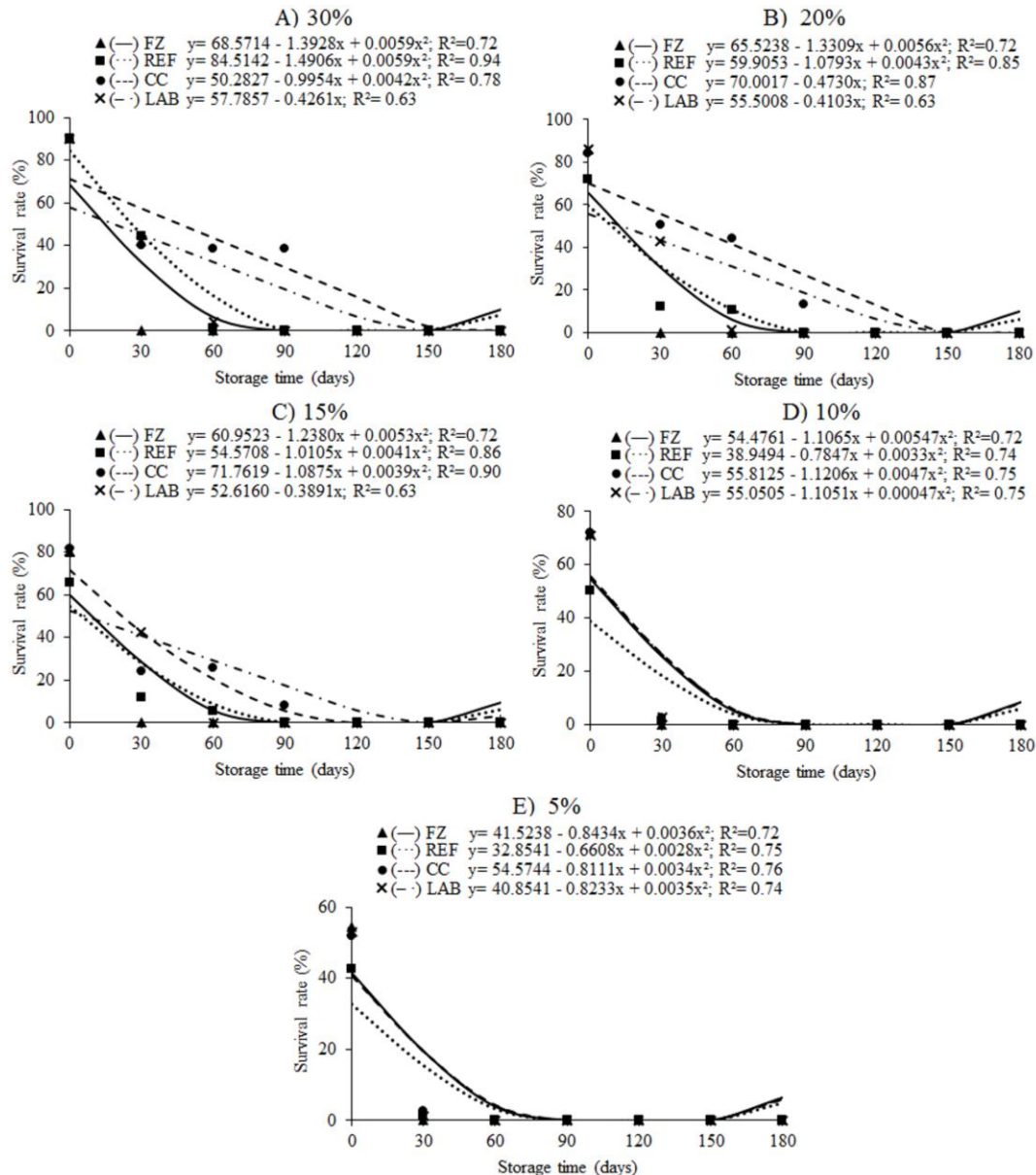
Figure 3. Survival rate of primary root of *C. adamantium* seedlings stored at water contents of 30 (A), 20 (B), 15 (C), 10 (D) and 5% (E), environmental conditions (LAB - laboratory (25°C), CC - cold and dry chamber (16°C), REF- refrigerator (8°C), and FZ - freezer (-18°C), and storage times (0, 30, 60, 90, 120, 150 and 180 days).

Desiccation of seeds to different water levels associated with storage environment and refrigerator conditions prevented the formation of shoots and primary roots after 60 days of storage (Figures 2 and 3). The desiccation of the seeds to water levels of 10 and 5% compromised seedling formation after 30 days of storage, regardless of storage conditions.

In relation to non stored seeds (storage time zero), we can emphasize that the relationship between shoot and primary root was proportional, reducing the value rate with seed desiccation. These high values in survival rates

are related to high incidence of normal seedlings, which are characterized by the presence of expanded cotyledons, hypocotyl, xylopodium, and well-defined primary root (Figure 4).

The reduction in the survival rate of primary roots was due to the high incidence of abnormal seedlings, which are characterized by the presence of expanded cotyledons, hypocotyl, xylopodium, and nonexistent or stunted primary root (Figure 4).

The anatomical characterization of the xylopodium in normal and abnormal seedlings show a normal pattern in
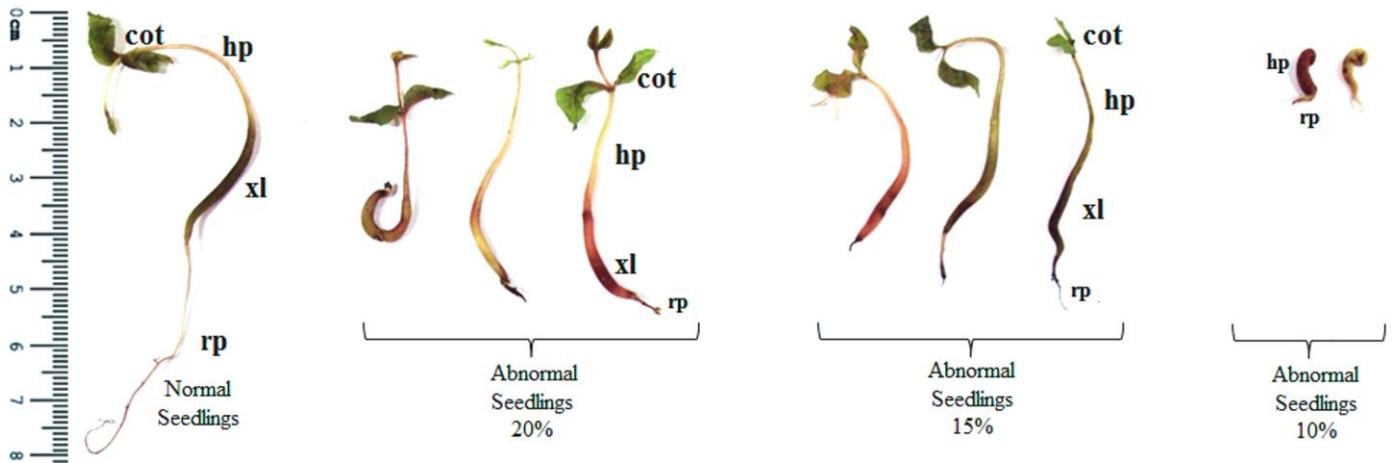
Figure 4. Overview of normal and abnormal *C. adamantium* seedlings (grown from seeds with water content of 20, 15 and 10%). cot, expanded cotyledons; hp, hypocotyl; xl, xylopodium; pr, primary root.
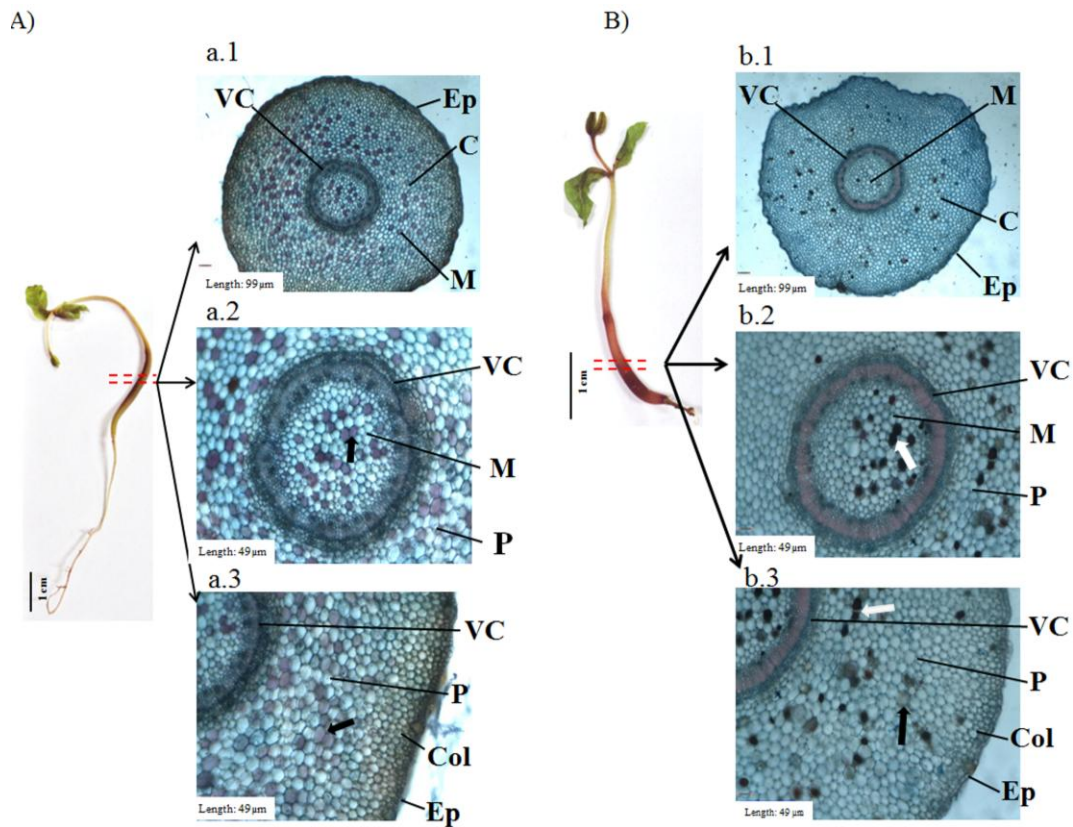
Figure 5. Cross section of xylopodium in primary stage of normal (a) and abnormal (b) seedling growth (M = medulla, C = cortex, P = parenchyma, Col = collenchyma, VC = vascular cylinder, Ep = epidermis, black arrow = lipids detected with Sudan III, and white arrow = phenolic compounds detected with ferrous chloride).

the early phase of development with well-defined regions such as the epidermis, cortical, and medullary regions. The innermost layer of the cortex is composed of lignified cells. The xylopodium in normal and abnormal seedlings shows lipids present in the cortex and medulla (Figures 5a and b). However, the phenolic compounds at this

Table 1. Histochemical tests of xylopodium in normal and abnormal seedlings *C. adamantium* from desiccated seeds with 20, 15 and 10% water contents.

Seedlings/water content	Classes of compounds			
	Lugol (starch)	Ferrous chloride (phenolic compounds)	Sulfuric acid (fructans)	Sudan III (lipophilic substance)
Normal	+	-	-	+
Abnormal / 20%	+	+	+	+
Abnormal /15%	+	+	+	+
Abnormal / 10%	+	+	+	+

(+) Positive and (-) negative.

stage of development were detected only in the cortex and medulla of abnormal seedlings.

In the histochemical test results, lugol used for identification of starch grains and Sudan III for lipids in general, showed positive reactions in the xylopodium of normal and abnormal seedlings (Table 1). Ferrous chloride used for identification of phenolic compounds and sulfuric acid (fructans) showed a strong positive reaction, especially in parenchyma cells of xylopodium in abnormal seedlings and a negative reaction in normal seedlings.

DISCUSSION

The formation of *C. adamantium* seedlings was influenced by desiccation, environmental conditions, and seed storage time. The dehydration of the seeds at different water contents associated with the storage conditions intensified the process of deterioration over time decreasing the rate in development of shoot and primary root to below 50% after 30 days of storage, except under the freezer condition.

The seeds packaged in semi-permeable plastic packaging in laboratory temperature allowed the exchange of water vapor between the seeds with high water content and the external environment, changing the level of hydration of seeds. These changes in water levels associated with the storage time influenced negatively the survival of the shoot and primary roots, confirming the recalcitrant behavior due to the sensitivity to drying and storing (Melchior et al., 2006; Scalon et al., 2013; Dresch et al., 2012, 2014).

The negative effects of desiccation, mainly at water contents below 15% associated with the storage times, resulted in the formation of abnormal seedlings, which had a missing or stunted primary root. Possibly, desiccation of these seeds associated with storage favored deterioration, which has as main cause lipids peroxidation (McDonald, 1999). Therefore, lipid peroxidation, occurring in the mitochondria of cells at the radicle end caused reduction in seedling growth in the most deteriorated seeds (Marcos Filho, 2005). The

occurrence of seedling abnormalities observed in the final stages of decay is determined by the death of important tissues in different regions of the seeds that cause severe damage to cellular metabolism and consequently disruption in seedling growth (Matthews, 1985; Marcos Filho, 2005).

The seedlings characterized as abnormal showed accumulation of phenolic compounds and fructans in parenchyma cells of the xylopodium, while the same does not occur in normal seedlings. The phenolic compounds result from lipid peroxidation due to seed deterioration (Marcos Filho, 2005). The damage caused by the deterioration may have contributed to the accumulation of phenolic compounds that triggered the malformation and growth of primary roots in seedlings. However, fructans accumulation may be associated with loss of membrane integrity during seed desiccation and storage. Fructans play an important role in osmotic regulation and in preventing membrane damage, maintaining the integrity and cell function, allowing not only the survival but also the growth even under conditions of low water availability, which occur due to either low temperature or lack of water in the environment (Brocklebank and Hendry, 1989; Demel et al., 1998; Vereyken et al., 2001).

Furthermore, the fructans are a source of energy or carbon reserve and therefore, like the phenolic compounds, are related to a species' tolerance to environmental stresses during growth and development, especially in the Cerrado area, a place where *C. adamantium* occurs naturally and where long droughts and fires can happen (Melo-de-Pinna and Menezes, 2003; Detmann et al., 2008). Several studies suggest that fructans provide the plants with resistance to drought and/or tolerance to cold (Livingston and Henson, 1998; Pilon-Smits et al., 1995, Van Den Ende et al., 2000). In studies of anatomy of the underground system in *Vernonia grandiflora* Less. and *V. brevifolia* Less. (Vernonieae, Asteraceae), was observed that the occurrence of these bud-forming underground systems, which stored reserve compounds, enabled these plants to survive throughout unfavorable environmental conditions in the Cerrado, such as dry season and

frequent fires in the winter (Hayashi and Appezzato-da-Glória, 2007).

The presence of fructans and phenolic compounds demonstrates the adaptive mechanism of the species in response to seed desiccation and storage temperature. In surveys conducted in the Cerrado area, it has been found that many species have underground storage organs that accumulate large amounts of fructans (Figueiredo-Ribeiro et al., 1986; Tertuliano and Figueiredo-Ribeiro, 1993; Hayashi and Appezzato-da-Glória, 2007; Appezzato-da-Glória and Cury, 2011).

The results obtained in this study are in agreement with our initial hypothesis that desiccation and storage does not affect the morphoanatomy and histochemistry of *C. adamantium* seedlings. It is worth noting that after the evaluation period of seedling survival, the emergence of secondary roots (data not shown) in abnormal seedlings was observed, reinforcing the information from the literature that fructans confer tolerance under stress conditions, such as desiccation and seed storage temperatures, thereby ensuring the survival of seedlings.

Future work should be conducted to assess the development of the root system and its implication in seedling production from seeds that are desiccated and stored at tolerable water levels in germplasm banks.

Thus, we conclude that the desiccation and storage of seeds affects seedlings formation by preventing normal development of the primary root and shoot structures. The deleterious effects of desiccation associated with storage triggers the onset of phenolic compounds and fructans in abnormal *C. adamantium* seedlings.

Conflict of Interest

The authors declare they have no conflict of interest.

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Full Length Research Paper

Biological parameters and parasitism capacity of *Telenomus remus* Nixon (Hymenoptera: Platygastridae) reared on natural and factitious hosts for successive generations

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Factitious hosts are largely used in parasitoid production. However, changes in parasitism capacity may happen when hosts are switched. Therefore, the ability of a parasitoid species to be reared on factitious host and still keep high level of parasitism on the natural target pest after successive rearing can determine parasitoid quality and must be investigated. Thus, we evaluated *Telenomus remus* parasitism on *Corcyra cephalonica* eggs compared with its natural host, *Spodoptera frugiperda* eggs, for different generations. After being reared on *C. cephalonica*, *T. remus* parasitism on *S. frugiperda* was evaluated to measure different *T. remus* biological parameters and parasitism capacity (parasitoid quality). Gradual increase in *C. cephalonica* eggs parasitized was observed over the generations, stabilizing on generation F_7. The number of parasitized *C. cephalonica* eggs was similar among generations (from generation F_7 to F_{19}). Taking the lifetime parasitism into consideration, parasitism capacity is similar from *T. remus* reared on *S. frugiperda* eggs from those reared on *C. cephalonica* eggs (generation F_{19}). When laboratory-produced *T. remus* on *C. cephalonica* eggs was exposed to the natural host, parasitism was higher on F_5 generation and stable from generations F_5 to F_{19}. Therefore, parasitoids did not lose their ability to parasitize eggs of natural host assuring good quality of the laboratory-produced parasitoid using *C. cephalonica* eggs as factitious host.

Key words: *Spodoptera frugiperda*, *Corcyra cephalonica*, pre-imaginal condition, egg parasitoid, biological control.

INTRODUCTION

The egg parasitoid *Telenomus remus* Nixon (Hymenoptera: Platygastridae) is an effective biological control agent for various pest species of the genus *Spodoptera* Guenée (Lepidoptera: Noctuidae) (Pomari et

al., 2012), mainly due to its high reproductive capacity (Cave 2000; Bueno et al., 2008). Despite all of its favorable features for biological control, currently this parasitoid is only reared on a small scale due to both difficulties and costs inherent in rearing it on its natural host, *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) (Pomari-Fernandes et al., 2014). Among the difficulties, *S. frugiperda* cannibalism stands out (Chapman et al., 2000). It makes necessary to rear the caterpillar in individual vials as a way to decrease the pre-imaginal mortality (Chapman et al., 2000) consuming a great deal of time and resources (Perkins, 1979) what consequently raises the costs of the parasitoid rearing.

Therefore, this parasitoid has been only used for experimental purposes against *S. frugiperda* and other lepidopterans or released in small acreages as for example in Venezuela (Ferrer, 2001). In order to increase this parasitoid use in agriculture worldwide, an effective and inexpensive mass-rearing method for *T. remus* in bioindustries is crucial what could be done using a factitious host. Parasitoids can even be reared in non-preferred hosts since they are adequate to promote acceptable development of insects (Parra, 1997). In this context, *Corcyra cephalonica* (Stainton, 1865) (Lepidoptera: Pyralidae) was chosen as a factitious host candidate for *T. remus* since eggs of this moth has been extensively used in China for the production of other eggs parasitoids such as *Trichogramma* spp. (*Bernardi* et al., 2000) and it had previously been pointed out as a possible factitious host for *T. remus* (Kumar et al., 1986). Moreover, *C. cephalonica* is a stored-product moth, which can easily be reared in laboratory on a large scale (*Bernardi* et al., 2000), possibly at lower costs compared to *S. frugiperda* for which its rearing is considered to be not practical and very time and resource consuming (Perkins, 1979). These are essential requirements to enable efficient massive production of natural enemies in the laboratory.

Nevertheless, it is important to emphasize that in order to establish and keep a parasitoid rearing on factitious host assuring high quality of produced parasitoid, parasitism capacity and development of the parasitoid on the selected factitious host must be taken into consideration and further analyzed. Moreover, release success of this parasitoid, produced on the factitious host, depends on the knowledge of the bioecological characteristics of the produced parasitoid and on its interaction with the targeted host in the field (Bourchier and Smith, 1996) which can be a different species from the host used in the laboratory rearing. In such example, changes in the foraging behavior and parasitism may happen when hosts are switched.

Parasitism behavior changes happens because it can be innate, as a result of the standards set in the genotype of the species (pre-imaginal conditioning) or present patterns that can be learned as a result of experience gained during foraging and parasitism. Many parasitoids are able to increase their search capacity due to experiences in a particular environmental situation or host species. Associating the signs learned during parasitism or during development, parasitoid female can readily locate and parasitize its host with greater efficiency and speed (Corbet, 1985, Nurindah et al., 1999), a phenomenon known as α - conditioning or associative learning (Vinson, 1998; Nurindah et al., 1999).

Then, considering possible changes in the parasitoid biology that can endanger its field efficacy after being rearing on factitious hosts for many generations, it is important to validate the use of this laboratory-produced *T. remus.* Therefore, laboratory studies investigating comparative parasitoid biology and its parasitism are needed (Hassan, 1997; Scholler and Hassan, 2001). Thus, the objective of this research was to evaluate whether it is possible to use the species *C. cephalonica* as alternate host for mass rearing of *T. remus*. Specifically, *T. remus* biology and parasitism capacity on the factitious host *C. cephalonica* was compared with that on its natural host, *S. frugiperda* for different generations. Moreover, after being reared on *C. cephalonica* eggs, *T. remus* parasitism on target pest, *S. frugiperda*, was evaluated in order to guarantee quality control of the laboratory-produced parasitoid. This research generated information which would allow future mass production of the parasitoid in the laboratory as well as the use of this egg parasitoid in extensive biocontrol programs.

MATERIALS AND METHODS

Biological characteristics of *T. remus* reared on eggs of *C. cephalonica* eggs for different generations. The experiment was conducted in a completely randomized design with 12 treatments (*T. remus* reared on eggs of *C. cephalonica* for different generations – F_0, F_1, F_2, F_3, F_4, F_5, F_6, F_7, F_8, F_9, F_{13}, and F_{19} generations) and 6 replicates (each replicate consisting of five individualized females) in a climatic chamber, adjusted at temperature of 25±2°C, relative humidity of 80±10% and photoperiod of 14/10 h (L/D). *T. remus* reared on *S. frugiperda* eggs and exposed to parasitism on *C. cephalonica* eggs made F_0 generation, then F_1 generation was the first generation of parasitoid reared on *C. cehphalonica* eggs and successively afterwards.

T. remus females (up to 24 h old) from *C. cephalonica* eggs of different generations were placed in individual vials, each containing a honey droplet (around 100 microliters of honey). Approximately 100 eggs of unviable *C. cephalonica* (up to 24 h) were glued onto a white Bristol board paper (2.5 cm x 5 cm), previously labeled with the respective treatments. These cards were placed in individual vials, each containing a single *T. remus* female, and sealed with Polyvinyl Chloride (PVC) film. *T. remus* was allowed to parasitize for 24 h. After 24 h, the cards were removed from the tubes and transferred into new glass tubes until the emergence of adults. For each parasitoid generation (treatment), the following biological parameters were observed: number of parasitized eggs, parental female longevity (days), duration of egg-adult period (days), emergence percentage (viability) and sex ratio. To determine the duration of the egg-adult period, daily observations from emergence to the adult stage of *T. remus* were made.

Parasitism capacity of *T. remus* on *C. cephalonica* eggs compared to *S. frugiperda* eggs. The experiment was conducted in a completely randomized design with five best treatments from the previous experiment (*T. remus* reared on eggs of *C. cephalonica* for different generations – F_0, F_8, F_{13}, and F_{19} parasitizing *C. cephalonica* eggs and *T. remus* reared on eggs of *S. frugiperda* parasitizing *S. frugiperda* eggs) and 6 replicates (each replicate consisting of five individualized females) in a climatic chamber, set at a temperature of 25±2°C, relative humidity of 80±10% and photoperiod 14/10 h (L/D).

Individual mated *T. remus* (newly emerged: ≤ 24 h old) were placed into separate glass tubes (12 mm Ø x 75 mm tall) covered with PVC film. Droplets (around 100 microliters each) of pure honey on the walls of the glass tubes were offered to feed the females. Thirty glass tubes (6 replications of 5 females each) were prepared for each treatment. Around 100 eggs of *C. cephalonica* (≤ 24 h old), from the host colony, were glued onto a white Bristol board paper (2.5 cm x 5 cm). Similarly, 100 eggs of *S. frugiperda* (≤ 24 h old) were glued onto different cards also made of white Bristol board paper (2.5 cm x 5 cm). Each paper was previously labeled with the respective treatments. Then, these cards, having the eggs, were offered for parasitism for 24 h. *C. cephalonica* eggs were submitted to a process of making them unviable by exposure to ultraviolet radiation for 30 min (Stein and Parra, 1987) previously to parasitism. The cards (with the eggs) were replaced daily until the death of the female parasitoid. The eggs daily removed from the glasses were maintained inside the same controlled environmental chamber under the controlled condition until the emergence of the parasitoids. The evaluated parameters were the number of parasitized eggs per day (daily parasitism), lifetime parasitism, and parental female longevity.

Biological characteristics of *T. remus* reared on *C. cephalonica* eggs for different generations and exposed to parasitism for 24 h on *S. frugiperda* eggs. The experiment was conducted in a completely randomized design with five treatments (*T. remus* reared on eggs of *C. cephalonica* for different generations – F_0, F_8, F_{13}, and F_{19} and *T. remus* reared on eggs of *S. frugiperda* as control) and four replicates (each replicate consisting of five individualized females) in a climatic chamber, set at temperature of 25±2°C, relative humidity of 80±10% and photoperiod of 14/10 h (L/D), according to the methodology described by Pomari et al. (2012) similarly to the experiment previously described. Treatments were females of *T. remus* reared on eggs of *C. cephalonica* for five different generations (F_5, F_6, F_8, F_{13}, and F_{19}) exposed to parasitism for 24 h on *S. frugiperda* eggs.

T. remus females (up to 24 h old) from *C. cephalonica* eggs of different generations (F_5, F_6, F_8, F_{13}, and F_{19}) were placed in individual vials, each containing a honey droplet. Approximately 100 eggs of *S. frugiperda* (up to 24 h) were glued onto a white Bristol board paper (2.5 cm x 5 cm), previously labeled with the respective treatments. These cards were placed in individual vials, each containing a single *T. remus* female, and sealed with PVC film. *T. remus* was allowed to parasitize for 24 h. After 24 h, the cards were removed from the tubes and transferred to new glass tubes until the emergence of adults. For each parasitoid generation (treatment), the following biological parameters were observed: number of parasitized eggs, parental female longevity (days), duration of egg-adult period (days), emergence percentage (viability) and sex ratio. To determine the duration of the egg-adult period, daily observations from emergence to the adult stage of *T. remus* were made.

Statistical analysis

Prior to ANOVA, the experimental results were submitted to exploratory analysis to test the normality of the residuals (Shapiro and Wilk, 1965), the homogeneity of variance of the treatments (Burr and Foster, 1972), and the additivity of the model. Means were compared using the Tukey test (5% error probability) through the SAS statistical analysis program (SAS Institute, 2001).

RESULTS

Biological characteristics of *T. remus* reared on eggs of *C. cephalonica* eggs for different generations. The number of *C. cephalonica* parasitized eggs differed over the parasitoid generations ($F_{11, 36} = 2.79$, $P < 0.0001$) (Table 1). There was a gradual increase in parasitism (%) from generation F_0 up to F_7 ranging from 2.98 parasitized eggs to 62.88 parasitized eggs in 24 h of parasitism, respectively (Table 1). There was similar parasitism (%) from generation F_7 (62.88%) to F_{19} (61.5%) (Table 1).

The average length of the egg-adult period (days) differed between generations ($F_{11, 36} = 36.96$, $P < 0.0001$), being initially lower (F_0 to F_2) with an increase of 1 or 2 days in the other evaluated generations (Table 1). Egg-adult period (days) was from 12 to 13.2 days in the first 3 generations (F_0 to F_2) and from 14 to 15 days from F_3 to F_{19} (Table 1). Parasitism viability (%) was greater than 78% in all evaluated generations (Table 1). It was similar and around 80% from F_0 to F_4 and increased to around 90% from F_5 to F_{19} ($F_{11, 36} = 13.94$, $P < 0.0001$). Sex ratio did not differed among the evaluated generation ($F_{11, 36} = 1.33$, $P = 0.2510$). Even though parental female longevity changed in the different generations ($F_{11, 36} = 9.16$, $P < 0.0001$), the observed variation seemed randomly and no patter could be observed (Table 1).

Parasitism capacity of *T. remus* on *C. cephalonica* eggs compared to *S. frugiperda* eggs. The lifetime number of parasitized *C. cephalonica* eggs by *T. remus* reared on *C. cephalonica* eggs increased over the generations ($F_{4, 20} = 38.30$, $P < 0.0001$). The higher amount of parasitized *C. cephalonica* eggs was found at generation F_{19}. These values were similar to the control treatment, which are parasitoid from natural host (*S. frugiperda* eggs) parasitizing the same natural host (*S. frugiperda* eggs) (Table 2). More than 80% of this lifetime parasitism of *T. remus* on eggs of *C. cephalonica* from generations F_{13} (Figure 1D) and F_{19} (Figure 1E) was reached, respectively, at 4th and 6th days of parasitism. These is similar to *T. remus* from natural host (*S. frugiperda* eggs) parasitizing the same natural host (*S. frugiperda* eggs) (Figure 1A) in which 80% of lifetime parasitism was reached at the 4th day. *T. remus* from generations F_0 and F_8 on *C. cephalonica* eggs took longer to reach 80% of lifetime parasitism, 10 and 9 days, respectively (Figure 1B and C). The number of parasitized eggs per day varied with parasitoid generations and hosts, but it was higher on the first 24 h on both studied variables (host and generation). In these result it is important to emphasize for all treatments parasitoid decreased the number of eggs daily parasitized on the studied host as a function of the time of parasitism (Figure 1A and E).

Table 1. Biological parameters of *T. remus* reared on *C. cephalonica* eggs for different generations and exposed to parasitism for 24 h on *C. cephalonica* eggs under controlled conditions [temperature of 25±2°C, relative humidity of 80±10% and photoperiod of 14/10 h (L/D)].

Generation	Number of *C. cephalonica* parasitized eggs[1]	Parental female longevity (days)[1]	Progeny		
			Egg-adult period (days)[1]	Parasitism viability (%)	Sex ratio
F_0	2.98 ± 0.22 [g]	10.5 ± 1.3 [c]	12.1 ± 0.4 [e]	82.58 ± 1.26 [bcd]	0.56 ± 0.03[ns]
F_1	5.63 ± 0.17 [fg]	13.3 ± 1.2 [bc]	13.2 ± 0.1 [d]	81.87 ± 1.23 [cd]	0.67 ± 0.04
F_2	8.55 ± 0.11 [efg]	10.4 ± 1.7 [c]	12.0 ± 0.0 [e]	80.17 ± 1.29 [cd]	0.65 ± 0.02
F_3	12.76 ± 0.17 [ef]	20.7 ± 1.5 [a]	14.0 ± 0.0 [c]	78.87 ± 1.94 [d]	0.65 ± 0.02
F_4	16.00 ± 0.24 [de]	19.0 ± 2.6 [ab]	15.0 ± 0.0 [a]	81.04 ± 2.39 [cd]	0.64 ± 0.04
F_5	22.19 ± 0.25 [d]	8.6 ± 0.7 [c]	14.0 ± 0.0 [c]	86.24 ± 0.75 [abc]	0.62 ± 0.02
F_6	38.72 ± 0.39 [c]	11.0 ± 0.2 [c]	14.6 ± 0.3 [abc]	90.26 ± 1.05 [a]	0.60 ± 0.04
F_7	62.88 ± 0.88 [ab]	9.0 ± 0.9 [c]	14.9 ± 0.1 [ab]	91.78 ± 1.59 [a]	0.66 ± 0.02
F_8	66.15 ± 0.95 [a]	10.0 ± 0.7 [c]	14.6 ± 0.1 [abc]	89.63 ± 0.32 [a]	0.68 ± 0.04
F_9	63.15 ± 0.82 [ab]	10.5 ± 0.2 [c]	14.1 ± 0.1 [bc]	88.46 ± 0.69 [ab]	0.62 ± 0.01
F_{13}	55.50 ± 0.97 [b]	11.6 ± 0.3 [c]	14.0 ± 0.0 [c]	89.64 ± 0.29 [a]	0.64 ± 0.01
F_{19}	61.50 ± 1.17 [ab]	10.8 ± 0.3 [c]	14.1 ± 0.1 [bc]	90.65 ± 0.14 [a]	0.65 ± 0.03
p	<0.0001	<0.0001	<0.0001	<0.0001	0.2510
F	2.79	9.16	36.96	13.94	1.33
DF$_{error}$	36	36	36	36	36
CV(%)	10.26	9.72	2.32	2.94	8.88

Means (Mean ± Standard Error) follow by the same small letters in the columns are not statistically different (Tukey test, P≤0,05). [1]Original data followed by analysis performed on data transformed into $\sqrt{X}$. [ns]ANOVA non-significant.

Table 2. Lifetime number of parasitized eggs, parasitism viability, sex ration and parental female longevity of *T. remus* reared on different hosts/generations under controlled conditions [temperature of 25±2°C, relative humidity of 80±10% and photoperiod of 14/10 h (L/D)].

Treatment	Lifetime number of parasitized eggs	Parental female longevity (days)	Progeny	
			Parasitism viability (%)	Sex ratio
1) *T. remus* from *S. frugiperda* eggs on *S. frugiperda* eggs	140.8 ± 10.9 [a]	8.3 ± 0.8 [b]	87.8 ± 1.6 [a]	0.67 ± 0.02 [b]
2) *T. remus* from *S. frugiperda* eggs on *C. cephalonica* eggs – generation F_0	30.3 ± 4.2 [c]	13.1 ± 0.4 [a]	15.0 ± 1.9 [c]	0.69 ± 0.02 [b]
3) *T. remus* from *C. cephalonica* eggs on *C. cephalonica* eggs – generation F_8	51.47 ± 7.71 [c]	15.3 ± 0.7 [a]	75.0 ± 3.0 [b]	0.75 ± 0.01 [a]
4) *T. remus* from *C. cephalonica* eggs on *C. cephalonica* eggs – generation F_{13}	107.4 ± 6.5 [b]	13.5 ± 0.3 [a]	77.9 ± 0.9 [b]	0.71 ± 0.01 [ab]
5) *T. remus* from *C. cephalonica* eggs on *C. cephalonica* eggs – generation F_{19}	122.4 ± 7.5 [ab]	13.5 ± 0.3 [a]	70.6 ± 1.2 [b]	0.69 ± 0.01 [ab]
p	<0.0001	<0.0001	<0.0001	0.0146
F	38.30	24.69	238.90	4.24
DF$_{error}$	20	20	20	17
CV (%)	18.92	9.21	6.39	4.14

Means (Mean ± Standard Error) follow by the same small letters in the columns are not statistically different (Tukey test, P≤0,05). [ns]ANOVA non-significant.

Parasitism viability was higher for the progeny of parasitoid from *S. frugiperda* eggs parasitizing *S. frugiperda* eggs (control treatment) compared to the other treatments (*T. remus* reared on eggs of *C. cephalonica* for different generations – F_0, F_8, F_{13}, and F_{19}) which did not differ among themselves ($F_{4, 20}$ = 238.90,
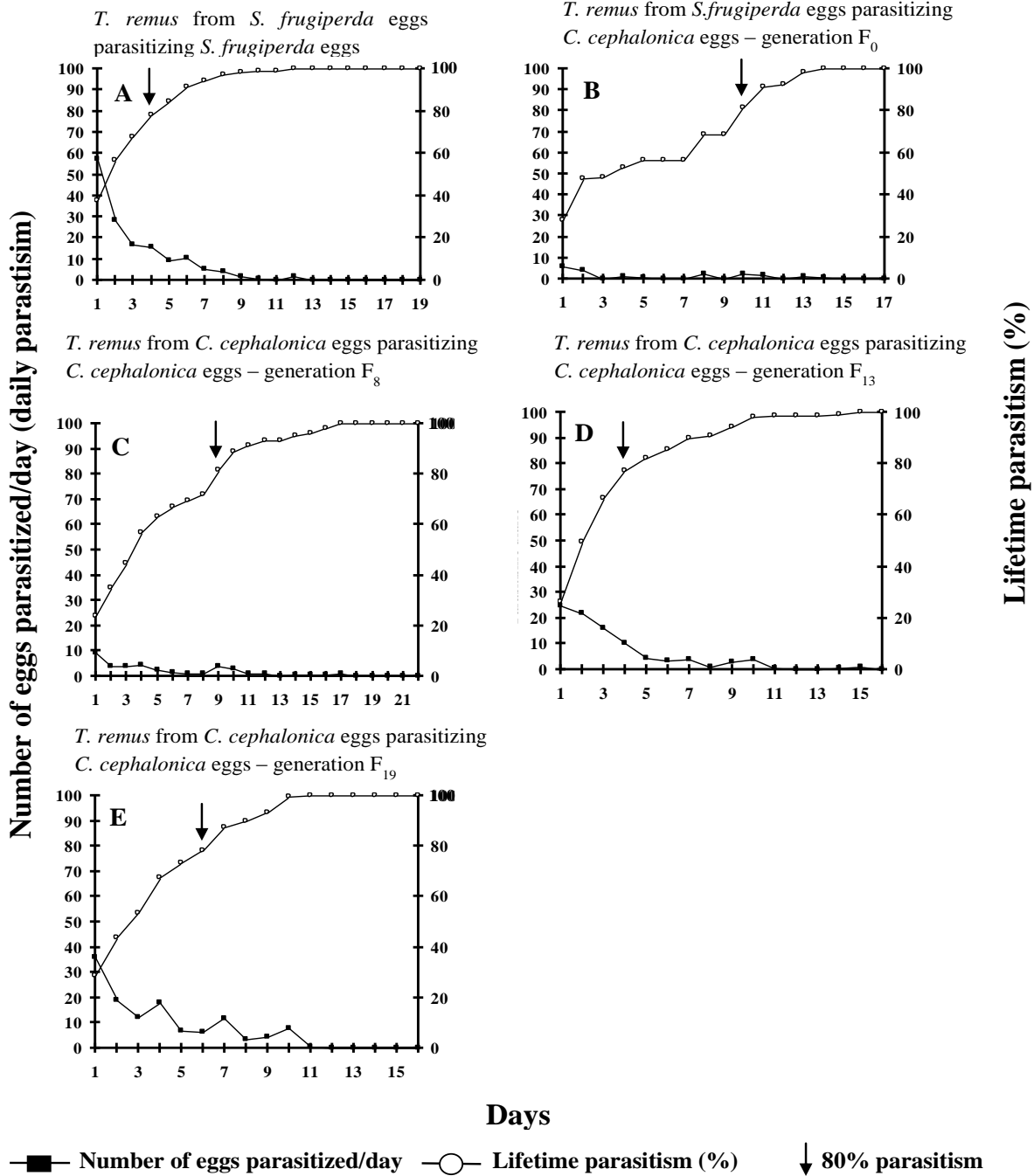
Figure 1. Number of eggs parasitized per day and lifetime parasitism (%) of *S. frugiperda* and *C. cephalonica* eggs by *T. remus* reared on different hosts/generations under controlled conditions [temperature of 25±2°C, relative humidity of 80±10% and photoperiod of 14/10 h (L/D)].

P <0.0001). *T. remus* from *S. frugiperda* eggs parasitizing *S. frugiperda* eggs presented 87.8% of parasitism viability while these values varied from 70.6 to 77.9% for *T. remus* reared on *C. cephalonica* eggs and parasitizing *C. cephalonica* eggs (Table 2). The sex ratio of progeny differed among treatments ($F_{4, 17} = 4.24$, *P* = 0.0146). However, all of them were higher than 0.6 (Table 2),

values greater than 67% of females, while female longevity ($F_{4, 20} = 24.69$, *P* <0.0001) was higher with eggs from *C. cephalonica*, regardless of generation (Table 2).

Biological characteristics of *T. remus* reared on *C. cephalonica* eggs for different generations and exposed to parasitism for 24 h on *S. frugiperda* eggs. The number of *S. frugiperda* parasitized eggs were similar among F_6,

Table 3. Biological parameters of *T. remus* reared on *C. cephalonica* eggs for different generations and exposed to parasitism for 24 h on *S. frugiperda* eggs under controlled conditions [temperature of 25±2°C, relative humidity of 80±10% and photoperiod of 14/10 h (L/D)].

Generation	Number of *S. frugiperda* parasitized eggs[1]	Parental female longevity (days)	Progeny		
			Egg-adult period (days)[1]	Parasitism viability (%)[1]	Sex ratio[1]
F_5	170.10 ± 9.85 [a]	15.75 ± 0.90[a]	10.00 ± 0.00[c]	88.44 ± 5.32[ab]	0.40 ± 0.04[b]
F_6	65.18 ± 10.08[b]	13.50 ± 0.98[a]	11.59 ± 0.14[a]	73.16 ± 5.91[b]	0.63 ± 0.03[a]
F_8	81.65 ± 7.00[b]	7.15 ± 0.41[b]	11.25 ± 0.14[ab]	91.14 ± 2.66[a]	0.66 ± 0.01[a]
F_{13}	49.45 ± 6.30[b]	7.85 ± 0.79[b]	11.00 ± 0.00[b]	96.92 ± 2.22[a]	0.75 ± 0.03[a]
F_{19}	51.60 ± 6.89[b]	6.60 ± 0.55[b]	11.10 ± 0.00[b]	97.40 ± 0.72[a]	0.72 ± 0.03[a]
p	<0.0001	<0.0001	<0.0001	0.0033	<0.0001
F	37.40	30.25	51.07	6.40	17.54
DF_{error}	15	15	15	15	15
CV(%)	19.58	14.90	1.51	8.71	10.34

Means (Mean ± Standard Error) follow by the same small letters in the columns are not statistically different (Tukey test, P≤0.05). [ns]ANOVA non-significant.

F_8, F_{13}, and F_{19} generations (Table 3). Differences were reported for egg-adult period ($F_{4, 15}$ = 51.07, *P* <0.0001) among the tested generations. For example, F_6 and F_8 generations took little longer (less than 2 days) to complete immature development compared to F_5 generation, which had the faster development (10.0 days) among the tested generations (Table 3). Parasitism viability (%), which is the percentage of parasitized eggs that presented parasitoid emergence, differed among parasitoid generations ($F_{4, 15}$ = 6.40, *P* = 0.0033), but was only smaller than 80% for parasitoid generation F_6. Generations F_8, F_{13}, and F_{19} presented values above 90% (Table 3).

Regarding to sex ratio ($F_{4, 15}$ = 17.54, *P* <0.0001) differences were only noted from generation F_5 (0.40) to F_6 (0.63). Generations F_6, F_8, F_{13}, and F_{19} did not differ among themselves and all presented sex ratio higher than 0.5 (Table 3). Parental female longevity also differed among parasitoid generations ($F_{4, 15}$ = 30.25, *P* <0.0001). Longevity was higher at F_5 and F_6 generations presenting 15.75 and 13.5 days, respectively. The values decreased for F_8, F_{13}, and F_{19} generations in which parental female longevity was 7.15, 7.85, and 6.60 days, respectively (Table 3).

DISCUSSION

The observed increase in *T. remus* parasitism over the generations reared on *C. cephalonica* eggs might suggest parasitism behavior changes as a result of selection of *T. remus* genotypes which are able to parasitize the factitious host. This learning process occurs in different parasitoid species at the time of emergence and may influence future host preference (Corbet, 1985; Hérard et al., 1988; Vet and Groenewold,

1990) which would mean the existence of pre-imaginal conditioning. Different parasitoid species are able to increase their parasitism in non-preferable hosts due to past experiences (Corbet, 1985; Nurindah et al., 1999). Associating the signs learned during parasitism, *T. remus* reared on *C. cephlonica* eggs over different generations could more readily locate and parasitize this factitious host with greater efficiency and speed compared to *T. remus* reared on *S. frugiperda* eggs, a phenomenon known as α - conditioning or associative learning (Vinson, 1998; Nurindah et al., 1999). Regardless of the form of learning exerted by the parasitoid through the generations, Kumar et al. (1986) reported that *T. remus* development in eggs of *C. cephalonica* was low at first generations increasing up to 100% parasitism on the seventh generation, which is similar to our results ratifying the existence of a adapting process in this parasitoid species over successive generations in order to develop on the factious host egg.

These conclusions differ from Goulart et al. (2011) who had previously reported non-preference of *T. remus* for *S. frugiperda* eggs after the parasitoid be reared on those eggs for several generations. Accordingly to these authors it would demonstrate that the host acceptance or preference behavior of the *T. remus* females could not be attributed to the pre-imaginal conditioning described by Cobert (1985) and Kaiser et al. (1989). However, it is important to point out that Goulart et al. (2011) compared *T. remus* preference among *Spodoptera* spp. eggs. Eggs from moths of the same genus might not exhibit enough differences for *T. remus* be able to differentiate among them. Peculiarities of each parasitoid host and their relative differences on the thickness of the chorion of the egg, especially the exochorion that is the most protein-rich layer, can affect not only the handling time and exploitation by egg parasitoids, but also the host

suitability (Pak et al., 1990). Eggs differences between *S. frugiperda* and *C. cephalonica* are certainly higher than when compared eggs inside *Spodoptera* genus. It could help to explain these differences observed when both findings are compared.

Our results, on the other hand, suggest that some generations are required for the parasitoid to adapt to *C. cephalonica* eggs. After this adaption process, this factious host could be used in *T. remus* massive rearing. However, it is important to confirm if the *T. remus* adapted to parasitize and develop on *C. cephalonica* eggs do not lose its capacity of parasitizing target field pests such as *S. frugiperda* eggs. Therefore, the number of *S. frugiperda eggs* parasitized by *T. remus* massive reared on *C. cephalonica* eggs for different generations is an important evaluation of the quality of this laboratory-produced parasitoid intended for field releases.

The number of parasitized *S. frugiperda* eggs in 24 h and the lifetime parasitism indicates a parasitism increase (recovery) after the decrease observed in the first 24 h. It is also important to observe that this noted lifetime parasitism is also similar those found when the parasitoid was reared on eggs of *S. frugiperda* reported in previous work (Pomari et al., 2012). Therefore, even though it might be necessary to release a higher number of *T. remus* when the parasitoid is from factitious host, this laboratory-produced parasitoid, reared on *C. cephalonica* eggs, did not lose its capacity to control *S. frugiperda* eggs. It is true even when the eggs are laid on superposed layers. Similar findings were reported by Kumar et al. (1986) who found that *T. remus* reared by 75 generations on *C. cephalonica* eggs was always able to parasitize the natural host *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) over all tested generations.

Parental female longevity is also an important biological feature related to parasitoid success in the field. Longer longevity is desirable and could mean a higher parasitism or at least a longer time in which the crop is protected against the target pest. It is correlated with the energy expenditure required for parasitism (Almeida, 2004) which can differ depending upon the host used. Differences in this parameter, when *T. remus* was exposed to the different hosts and generations, may indicate a higher or lower energy expense for the parasitism. This can explain the usually observed smaller lifetime directly related to higher parasitism rates. Moreover, this lower energy expenditure can be coupled with the fact *C. cephalonica* eggs be offered isolate in single layer, which may have led to lower metabolic rate, consequently increasing female longevity compared to *S. frugiperda* eggs that are laid on superposed masses.

In addittion, more active females can increase body temperature due to the parasitism activity. Female longevity is usually impacted by temperature being lower at warmer environment (Gerling, 1972). This relationship was previously reported by Almeida (2004), evaluating

Trichogramma atopovirillia (Oatman and Platner, 1983) (Hymenoptera: Trichogrammatidae) parasitism. According to them higher energy expenditure of females is due to parasitism, firstly with the introduction of the ovipositor and oviposition, followed by a review of the host by contact with the antennas (Almeida, 2004). Besides these factors, one should take into account that *T. remus* females have the maximum number of eggs in their ovaries between 2 to 3 days of age (van Welzen and Waage, 1987), and produce more than 76% of their offspring during the first 5 days of adulthood (Schwartz and Gerling, 1974). Thus, longevity of parental females, although important, is not a determining factor between the hosts, since parasitoids from both hosts lived longer than 5 days.

T. remus parasitism period (which is the time for how long *T. remus* females are active) might differ from female longevity. Parasitism period varies due to differences in temperature (Reznik and Vaghina, 2006), hosts (Reznik et al., 2001), or parasitoid species/strain (Pratissoli and Parra, 2000; Pizzol et al., 2010) and can influence the success of biological control programs using egg parasitoids (Smith 1996). Also, whether parasitism is more active in the first days of life or evenly distributed throughout adulthood is an important characteristic to be considered in choosing the best parasitoid release strategy (Bueno et al., 2010). Our results showed that parasitism activity of *T. remus* always peaked in the first 24 h of parasitism regardless of host species or generation, similarly to what has been reported for this species when attacking *S. frugiperda* (Hernández and Díaz, 1996; Bueno et al., 2010).

The concentration of the parasitism activity in the first days of life is not a characteristic only common to *T. remus*, but also to other egg parasitoids, which need to quickly find their hosts and assure the allocation of their progeny. Oviposition peak of different species of egg parasitoids from the genus *Trichogramma*, for example, have been already reported in the literature on the first day after adult emergence (Bai et al., 1992; Volkoff and Daumal, 1994). This is usually a consequence of most of these parasitoids to have the capacity to store a full complement of mature eggs in the ovaries or oviducts and complete oogenesis either before or shortly after adult emergence (pro-ovigenic parasitoids) (Mills and Kuhlmann, 2000) and, thus, adults emerge ready to lay eggs. In contrast, other studies indicate that some parasitoid species emerge with an egg load that accounts for only a fraction of their potential parasitism, which exceeds their capacity to store mature eggs in their ovaries or oviducts (synovigenic parasitoids) (Kuhlmann and Mills, 1999).

Parasitoid development (egg-adult period) is another biological trait important to consider when comparing host suitability. This parameter is dependent on different biotic and abiotic factors (Pomari et al., 2012). Nutritional requirements from *T. remus* immature development

determines the need for a longer or shorter period of development. Most *T. remus* egg-adult periods in this study did not differ more than 2 days apart, indicating that these biological parameter is not affected over the different *T. remus* generations on *C. cephalonica* eggs. Moreover, similar egg-adult period (days) had been reported when *T. remus* parasitized *S. frugiperda* eggs (Bueno et al., 2008; Pomari et al., 2012). This is a clear indication that *T. remus* is adapted to parasitized *C. cephalonica* eggs. Its relationship has been demonstrated for other egg parasitoids such as *Trichogramma pretiosum* (Pratissoli and Parra, 2000; Bueno et al., 2009).

It is important to emphasize intrinsic characteristics of the egg as nutritional quality may be the key factor in the acceptance of the host and development of the parasitoid (Shipp et al., 1998; Pratissoli and Parra, 2001). Therefore, *C. cephalonica* seems to have enough nutritional quality for *T. remus* development. Other host eggs and/or parasitoid species may have different results for durations of the egg-adult period (Gerling, 1972; Gerling and Schwartz, 1974; Hernández and Díaz, 1996). The sex ratio is another important biological feature in biological control programs since greater proportion of females is desirable because they are responsible for parasitism (Navarro, 1998; Bueno et al., 2008). In this context, all tested generations supported more than 50% females indicating that the use of egg parasitoid *C. cephalonica* as factitious host in massive rearing does not affect the development of *T. remus* females. Additionally, the percentages of females obtained were similar to those observed for the host establishment and others of the same genus (Pomari et al., 2012). Thus, the smaller size of the *C. cephalonica* eggs in relation to the preferred hosts did not influence this biological parameter.

In conclusion, the results obtained with *T. remus* on eggs of *C. cephalonica* were satisfactory enough to state that egg from this stored-grain moth can be successfully used as *T. remus* factitious host. It is important to mention the parasitoid reared by successive generations in eggs of this factitious host has not lost its ability to parasitize eggs of the natural host. Although there was a decrease in the number of parasitized eggs, sex ratio and parasitism viability were not affected. The reduced number of parasitized eggs is not a problem since it can be circumvent with an increase in the number of parasitoids to be released. Also, parasitoids reared on factitious host can go throughout a generation on its target species from time to time in order to keep or even increase it parasitism aggressiveness. This method was used by Gautum (1994) using egg *Agrotis spinifera* (Hubner, 1808) (Lepidoptera: Noctuidae) as a factitious host for a generation/year to increase the effectiveness of *T. remus*, since these eggs were adults reared larger, longer-lived and more fertile than those obtained from *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae)

eggs. Nevetheless, massive *T. remus* rearing on *C. cephalonica* eggs is already a reality can be successfully perfomed since the adaption period of at least 13 generation is taken before starting releasing this parasitoids in the field.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Effect of Silicon and Nitrogen nutrition on major pest and disease intensity in low land rice

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Nutrition management is one of the most important factors for enhancing yield, but it may affect the response of rice plants to pests and diseases due, in part, to the change of microclimate under the rice plant canopy. Therefore, knowledge of the relationship of nutrition management and pests and diseases is an important component in setting up a high yield production system. The pest and disease control procedures used by farmers can as also include soil fertility management since these nutrition practices can impact the physiological susceptibility of crop plants to pest and diseases by affecting plant resistance. Silicon content is particularly important in pest and disease reduction in rice and certain rice genotypes are more efficient accumulators of silicon than others making them more resistant. In the absence of natural heritable resistance in rice varieties, resistance can be induced by alternate strategies to suppress certain pest and pathogens. Hence experiments were carried out at Jabugam Farm, Anand Agricultural University Anand Gujarat (India). Treatments were arranged in a factorial randomized block design with the silicon factor at four levels (0, 200, 400 and 600 kg ha^{-1}), four levels of nitrogen (0, 75, 100, and 125 kg ha^{-1}), and with three replicates. Indian improved and high – yielding rice variety, GR-13, was used. The overall yield ranged from 4991 to 6439 kg ha^{-1} with a mean of 5936 kg ha^{-1}. The plots which did not receive any fertilizer, that is, (N0 + Si0) exhibited the highest pest incidence (dead hearts, leaf folder and stem borer) and disease incidence (leaf blight, brown spot and grain discoloration) compared to other treatments which received both N and Si.

Key words: Nutrition management, conventional rice, silicon, yield, pest and diseases.

INTRODUCTION

Nutrition management is one of the most important factors for enhancing yield, but it may affect the response of rice plants to pests and diseases due, in part, to the change of the microclimate under the rice plant canopy. Therefore, knowledge of the relationship of nutrition management and pests and diseases is an essential component in setting up a high yield production system. The pest and disease control procedures adopted by farmers can also include soil fertility management since these nutrition practices can affect the physiological susceptibility of crop plants to pest and diseases.

Soils with high organic matter and active biological

activity generally exhibit good soil fertility. Studies have shown that the shift from organic soil management to chemical fertilizers has increased the potential of certain pest and diseases causing economic losses. Ramesh et al. (2005) reported that organic crops are more tolerant to insect attack, and organic rice is reported to have a thicker cell wall and lower levels of amino acid than conventional rice. Silicon is particularly effective against pest and diseases in rice and certain rice genotypes are more efficient accumulators of silicon than others making them more resistant (Winslow, 1992). In the absence of natural heritable resistance in rice varieties, resistance can be induced by alternate strategies to suppress certain pest and pathogens. Hence the present study was undertaken to study induced resistance and its impact on major pests and diseases of rice.

MATERIALS AND METHODS

The experiments were carried out at the Jabugam Farm, Anand Agricultural University Anand Gujarat (India), during *kharif* 2014. Geographically, Jabugam is situated at 22°17'37.70" North latitude, 73°46'41.02" East longitude with an elevation of 92 m above mean sea level. The climate of Jabugam region is semi-arid and sub-tropical with hot summer and cold winter. In this region, generally monsoon commences in the month of June and retreats from the end of September. Most of the rainfall is received from South-West monsoon currents. July and August are the months of heavy showers.

The field experiment was conducted in sandy clay loam to clay loam textured soils of rice growing areas. The nature of soil was acidic to neutral and pH ranged from 6.32 to 7.20. The soluble salts content was low to high and mean value was 0.43 ds m^{-1}. The available nitrogen, phosphorus, potassium and sulphur ranged from medium to high. Treatments were arranged in a factorial randomized block design with silicon factor at four levels (0, 200, 400 and 600 kg ha^{-1}) and four levels of nitrogen (0, 75, 100, and 125 kg ha^{-1}) consisting of 16 treatments with three replicates. Indian improved and high –yielding rice variety, GR-13, was used. To know the available silicon status of rice growing plots, representative soil samples were also collected and were estimated as per standard procedure given by Korndorfer et al. (2001). Grain and straw yields were recorded at harvest of rice crops. Recommended doses of phosphorus (50 kg ha^{-1}) were applied basally to all treatments in the form of single super phosphate (SSP). Nitrogen was applied as per the treatment schedules in the form of ammonium sulphate in three equal splits (1/3 basal, 1/3 at active tillering stage and 1/3 at panicle initiation stage). Silicon was applied in the form of calcium silicate (20% silicon content) as a basal application as per the treatment composition. Evaluation of the incidence of various pests *viz.,* the yellow stem borer (*Scirpophaga incertulas*) which causes dead hearts, and the rice leaf folder (*Cnaphalocrosis medinalis*) were recorded during tillering vegetative and reproductive phases by following standard procedures (Anonymous, 2007b). The disease incidence was assessed by recording the severity of leaf blight (*Xanthomonas oryzae*) and brown spot (*Helminthosporium oryzae*) during boot leaf, tillering and at harvest stage, whereas, sheath rot (*Sarocladium oryzae*) and grain discoloration (complex disease caused by fungi and bacteria) were recorded at harvest of the rice crop in accordance with a standard evaluation system by adopting a 0 to 9 scale and calculating percent disease intensity (PDI) as per Wheeler (1969). The analysis of variance for grain and straw yield,

pest and diseases was calculated using INDOSTAT software.

Statistical analysis

Experimental data were analyzed using analysis of variance method. Correlation and regression coefficients were worked out and the results are reported at 5 and 1% level of significance (Steel and Torrie, 1982).

RESULTS AND DISCUSSION

Effect of silicon and nitrogen on grain and straw yield

Data in Table 1 illustrate that application of N and Si had a significant effect on the seed yield of rice. The highest grain (6439 kg ha^{-1}) and straw yields (8892 kg ha^{-1}) recorded due to application of 125 kg N ha^{-1} and 600 kg Si ha^{-1} and were on a par with the yield obtained from other treatments receiving N at 100 and 125 kg ha^{-1} along with silicon at 200, 400 and 600 kg ha^{-1}. Even the treatments with N at 100 and 125 kg ha^{-1} without any silicon supplementation recorded significantly lower grain and straw yields of 4991 and 6539 kg ha^{-1}, respectively compared to the treatments which received silicon along with nitrogen (Table 1).

This might be due to the increased phosphate uptake that occurs with the application of silicon (Ma and Takahashi, 2002). But in the case of treatments which received no nitrogen and nitrogen at 75 kg ha^{-1}, the average yields were less than the treatments which received N at 100 kg ha^{-1} and 125 kg ha^{-1}. This may be due to the influence of conjunctive application of Si and N, which decreases percent spikelet sterility with increased Si levels (Snyder, 1991). Application of silica increased rice yield on histosols mainly due to the supply of plant available Si and not due to supply of other nutrients (Snyder, 1986). Similar to grain yields, the results revealed a significant influence of fertility levels as well as their interactions on rice straw yields. This higher straw yield could also be attributed to increased number of tillers per hill and plant height.

The dry matter production increased significantly with each increment in N and Si fertility level due to increased chlorophyll formation which ultimately improved photosynthesis in different rice soils of India (Singh et al., 2006). Nitrogen fertilization elicited an increase in this component which is associated to a greater availability of N since this nutrient is related to the formation of tissues (Matichenkov et al., 2001). The yield plateau observed at the highest level of N application could be due to the reason that increases in the number of stalks provided by increasing rates of N fertilization contributed to an increase in the number of leaves which could have caused shading, decreasing the area of active photosynthesis. Above all, excess N also prolongs the vegetative growth at the cost of reproductive growth, thus, diminishing the production of carbohydrates (Mauad et al 2003).

Table 1. Influence of different levels of nitrogen and silicon on grain yield (kg ha^{-1}) of rice under low land conditions.

Treatment (kg ha^{-1})		Yield (kg ha^{-1})	
		Grain	Straw
T_1	(N 0 + Si 0)	4991	6539
T_2	(N 0 + Si 200)	5494	7206
T_3	(N 0 + Si 400)	5556	7485
T_4	(N 0 + Si 600)	5594	7553
T_5	(N 75 + Si 0)	5487	7224
T_6	(N 75 + Si 200)	5901	7814
T_7	(N 75 + Si 400)	6086	8205
T_8	(N 75 + Si 600)	6283	8493
T_9	(N 100 + Si 0)	5818	7804
T_{10}	(N 100 + Si 200)	6185	8527
T_{11}	(N 100 + Si 400)	6249	8638
T_{12}	(N 100 + Si 600)	6357	8751
T_{13}	(N 125 + Si 0)	5741	7839
T_{14}	(N 125 + Si 200)	6350	8772
T_{15}	(N 125 + Si 400)	6405	8806
T_{16}	(N 125 + Si 600)	6439	8892
S.Em.±		161	215
C.D. (0.05)		466	620
CV %		9.43	9.26

Pest incidence

The data on the incidence of pests as influenced by different levels of N and Si in rice during the crop growth period is presented in Table 2. Three different pests viz., stem borer, dead hearts and leaf folder were observed at vegetative and panicle initiation stage, respectively. Among the fertility levels, the T_1 treatment which did not receive any N or Si levels recorded the highest incidence of stem borer and dead hearts as well as leaf folder as evidenced by stem borer, dead hearts and leaf folder, compared to other treatments which received both N and Si. An increase in the level of N increased the incidence of stem borer, dead hearts and leaf folder; which of course is common for rice (Savant et al., 1999). However, when N was integrated with Si, the incidence was decreased. Even among the treatment combinations, the treatments which received higher doses of Si in combination with N recorded lower incidence of stem borer, dead hearts and leaf folder compared to the treatments which received low Si content in combination with N.

The main cause for the death of insects due to silicon application was wearing of mandibles and main feeding organs of insects which resulted in functionless mandibles so that the insects of paddy die without food (Datnoff et al., 2001). There is increasing evidence for the necessity to modify the traditional view of silicon deposition in the cell walls as a purely physical process leading to mechanical stabilization (rigidity) of the tissue and acting as a mechanical barrier to pathogens. Silicon deposition is under rather strict metabolic and temporal control. Cell wall metabolites interact with silicic acid (forming ester bonds) leading to bulk deposition of silicon into the mature cell wall structure (Perry et al., 1987).

Panda et al. (1975) and Sasamoto (1961), reported that the larvae of the rice yellow stem borer, brown plant hopper, and leaf roller were unable to attack rice plants which became resistant because of high Si content in their stems; and a significantly negative correlation was shown between Si content and pest incidence. Savant et al. (1997) reported that plants with higher Si contents in their tissues had a higher level of resistance to rice pests. Several economically important insect pests such as stem borer, brown plant hopper, green leaf hopper have been suppressed by increasing the Si concentration in plants (Aziz et al., 2002).

Disease incidence

The data on the intensity of various diseases as influenced by different levels of N and Si in rice during the crop growth period is presented in Table 3. Diseases viz., leaf blight, brown spot and grain discoloration were observed and studied. Conjunctive use of Si and N on control of diseases (leaf blight, brown spot and grain discoloration) was observed at vegetative and panicle

Table 2. Influence of different levels of nitrogen and silicon on pest incidence under low land rice conditions.

Treatment (kg ha^{-1})		Pest incidence (%)		
		Dead hearts	Leaf folder	Stem borer
T_1	(N 0 + Si 0)	1.23	1.59	1.60
T_2	(N 0 + Si 200)	1.18	1.57	1.50
T_3	(N 0 + Si 400)	1.09	1.47	1.45
T_4	(N 0 + Si 600)	0.97	1.23	1.32
T_5	(N 75 + Si 0)	1.26	1.69	1.65
T_6	(N 75 + Si 200)	1.11	1.35	1.44
T_7	(N 75 + Si 400)	1.08	1.28	1.38
T_8	(N 75 + Si 600)	0.95	1.20	1.28
T_9	(N 100 + Si 0)	1.28	1.74	1.66
T_{10}	(N 100 + Si 200)	0.97	1.27	1.38
T_{11}	(N 100 + Si 400)	0.89	1.20	1.28
T_{12}	(N 100 + Si 600)	0.87	1.14	1.08
T_{13}	(N 125 + Si 0)	1.30	1.78	1.71
T_{14}	(N 125 + Si 200)	0.92	1.20	1.23
T_{15}	(N 100 + Si 400)	0.88	1.14	1.19
T_{16}	(N 125 + Si 600)	0.85	1.11	0.97
S.Em.±		0.03	0.04	0.05
C.D. (0.05)		0.08	0.11	0.13
CV %		8.72	9.35	11.70

Table 3. Influence of different levels of nitrogen and silicon on disease intensity during crop growth under low land rice conditions.

Treatment (kg ha^{-1})		Diseases intensity (%)		
		Leaf blight	Brown spot	Grain discoloration
T_1	(N 0 + Si 0)	0.224	0.554	0.929
T_2	(N 0 + Si 200)	0.182	0.511	0.906
T_3	(N 0 + Si 400)	0.158	0.401	0.819
T_4	(N 0 + Si 600)	0.149	0.181	0.647
T_5	(N 75 + Si 0)	0.241	0.570	0.943
T_6	(N 75 + Si 200)	0.160	0.361	0.778
T_7	(N 75 + Si 400)	0.136	0.209	0.691
T_8	(N 75 + Si 600)	0.114	0.152	0.513
T_9	(N 100 + Si 0)	0.263	0.601	1.055
T_{10}	(N 100 + Si 200)	0.140	0.182	0.614
T_{11}	(N 100 + Si 400)	0.127	0.145	0.594
T_{12}	(N 100 + Si 600)	0.117	0.139	0.479
T_{13}	(N 125 + Si 0)	0.271	0.625	1.073
T_{14}	(N 125 + Si 200)	0.132	0.162	0.540
T_{15}	(N 100 + Si 400)	0.123	0.138	0.515
T_{16}	(N 125 + Si 600)	0.107	0.123	0.464
S.Em.±		0.005	0.011	0.022
C.D. (0.05)		0.014	0.031	0.062
CV %		10.18	11.82	10.32

initiation stage respectively, during crop growth period. It was observed that increased levels of N alone increased the intensity of diseases. Disease intensity was significantly influenced by the different N and Si

combinations. Among the different N and Si level treatments, T_{10} (N_{100} + Si_{200}), T_{11} (N_{100} + Si_{400}), T_{12} (N_{100} + Si_{600}), T_{14} (N_{125} + Si_{200}), T_{15} (N_{125} + Si_{400}) and T_{16} (N_{125} + Si_{600}) recorded the lowest disease incidence compared to other treatments which received lower levels of nitrogen + silicon doses. However, there was significant reduction in diseases (leaf blight, brown spot and grain discoloration) as well as insect pests (stem borer, dead hearts and leaf folder) with increased levels of Si along with varied levels of N which ultimately resulted in increased grain yield. Promoter or carrier-induced silicon transportation into rice in relation to disease resistance has been investigated by Voleti et al. (2008).

They reported that simple amino acids, such as histidine, imidazole, glutamic acid, glycine and glutamine significantly enhanced the levels of $Si(OH)_4$ in the stem and resulted in 14 to 18% silicon transport into the leaf surface. Deren et al. (1994) concluded that the increase in rice yield with added Si was attributing to increased resistance against diseases such as brown spot, being negatively correlated with Si concentration in the plant tissue. In Brazil, Korndorfer et al. (1999) found that an increase in the Si application rate reduced grain discoloration from 46% in the control to 29% at the highest Si rate (960 kg Si ha^{-1}). It was also found that this difference corresponded to a 64 per cent reduction in grain discoloration.

It was previously reported that silicon has the capacity to reduce the severity of several important diseases of rice *viz.,* blast, brown spot, sheath blight, leaf scald, and grain discoloration (Seebold et al., 2001). The fungal hyphae and haustoria successfully infect the plant cell will break the cell wall through chemical and physical means. Formation of a physical barrier in epidermal cells by Si deposition may contribute to plant resistance against diseases and pests (Epstein, 1994).

Conclusion

The nitrogen and silicon have shown significant impact on the pest and disease incidence with higher doses reducing the incidence.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Some agroforestry potential for two tree legumes in the dense rainforests of Côte d'Ivoire: Characteristics of seed germination

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The Ivorian forest area has been declining because of his abuse. To reduce the negative impact of forest loss on natural resources, various reforestation projects are undertaken. The species used in these programs are usually rapidly growing exotic legumes. Unfortunately, these plants are very poorly adapted to the prevailing conditions and targets are rarely achieved. Hence, replacing them with native species should be considered. In order to meet this requirement, it would be necessary to select some taxa from the local flora with the same characteristics (such as high germination rate, rapid growth, etc.) and this study follows this pathway with research into the morphological characteristics and seed germination of two legumes (*Dialium guineense and Distemonanthus benthamianus*) and the survival rate of seedlings from germination. For this, fruits and seeds of these two species were collected, observed, described and put into germination. The results give 43.33% germination capacity among *D. guineense* and 67.77% in *D. benthamianus* when seeds do not undergo treatment. These rates can be improved by applying their specific treatments. A rate of 85% is obtained after scarification of the *D. guineense* seed. The rate of *D. benthamianus* increases to more than 88% after soaking in water. The different survival rates are estimated at over 80%. These plants may be used instead of exotics.

Key words: Legumes, germination rate, survival rate, seeds, young Ivory Coast plants.

INTRODUCTION

Ivory Coast gets most of its foreign currency from agriculture and the exploitation of wood resources, but faces the rapid loss of its forest cover. Where there were 16 million hectares of forest in 1960, the size dropped to 10,000 million hectares in year 2006 and is currently less than 2 million hectares (Anonymous, 2006), resulting in soil depletion and the loss of plant species, among others (Ake-Assi, 1988). Consequently, arable land and timber resources are becoming increasingly scarce and land

degradation and deforestation are major concern for the State. In its search for solutions, Ivory Coast has developed reforestation and agroforestry programs that use generally multiple exotic species whose essential features are rapid growth and the recoverability of soil fertility (Oszwald, 2005).

Many studies have demonstrated that the species of the *Leguminoseae* family are fast growing and have, in addition, the ability to protect, restore and enhance

soil fertility, due to symbiotic bacteria. The pioneer species most commonly used are trees and shrubs of the genus *Acacia* (Konate, 2007).

Two species are mainly used in Ivory Coast: *Acacia mangium* and *Acacia auriculaeformis*. Studies on these species do not yet predict the externalities that could impact upon and cause a hazard by their long-term presence, especially on biodiversity, ecology and the environment. Therefore, the use of local materials, as an alternative to exotic materials currently employed in the majority of reforestation projects in Côte d'Ivoire is necessary. Studies on their domestication are underway and they concern in particular the characteristics, growth and development of their seeds. It is in this context that the present work was undertaken, in relation to the morphological and physiological characterisation of the seeds of two species of socioeconomic interest, and which are commonly found in the forests of Southern Ivory Coast, namely, two *Caesalpinoideae* trees: *Dialium guineense* and *Distemonanthus benthamianus*.

MATERIALS AND METHODS

Location of the site

The study was carried out in the Abidjan region of Southern Côte d'Ivoire between latitudes 5°00' et 5°30' N and longitudes 3°50' et 4°10'W (Figure 1). The climate is Guinean, which is characterised by two dry seasons and two rainy seasons. The original vegetation was dense moist evergreen forest (Aubreville, 1959), but is now down to a fallow mosaic of all ages covered with herbaceous type vegetation with few primary or secondary forests such as the Banco and Anguédédou.

Plant material

The work focused on fruits and seeds of two species of common legumes in Ivorian forests, *D. guineense*, a food and medicinal plant, and *D. benthamianus*, a large multipurpose tree used for medicines, carpentry, etc. *D. guineense* is a species that is currently threatened due to its mode of exploitation, whereby trees are usually slaughtered during the picking of their edible fruits. *D. benthamianus* has a tendency to disappear because of its misuse and the destruction of its habitat to make way for crops.

Methodological approach

Harvesting technique for fruits and seeds

Phenological localisation and plotting: Prior to the harvest of fruits and seeds, parent plants were previously located via a roaming inventory and their phenology studied. Localisation was undertaken by making regular readings by browsing the study area. Whenever either of the two species was identified, we noted its geographical references using a global positioning system (GPS). Direct observation of individuals permitted the noting and monitoring of their phenology. In the fruiting state, fruit or seed were harvested following a methodology which will be described in the next section.

Harvest of fruits and grains: The harvesting technique for fruits

and seeds varies according to species. With *D. benthamianus*, mature pods are harvested directly from around the seeds after their fall. These are dried domestic fruits. The extraction of seed from its papery pods necessitates the use of scissors. In *D. guineense*, fruit is harvested directly from the trees. These are also domestic pods, being leathery and whose opening is possible only after strong pressure achieved with a hammer. The seeds are surrounded by an orange mealy pulp which is extracted from the pods before use.

Morphological characterisation of fruits and grains

The colour, shape and measurements of fruits and seeds were made by using a simple methodology (direct observations, measurements and weights) that can be repeated over time, and allow a smooth realisation of observations. Some phenotypic characteristics were observed using the naked eye (colour, geometry, etc.). The measured height and diameter of the bodies were made by using a calliper. The images were made by using digital photography equipment.

Treatment and sowing of seeds

Previously harvested seeds were sorted to eliminate waste and other non-viable seeds. The seeds that were clean in appearance and that would be used were stored in bags or conservation packets. For each species, the seeds were divided into three lots. The first was sown without pre-treatment. The seeds of the second batch were scarified before sowing. Those of the third batch were soaked in tap water for 24 h. Seedlings were cultivated in black bags with holes pierced into the sides in their basal part. They were 18 cm in height and 10 cm in diameter. The experimental design was a Fisher block with 3 treatments and 3 repetitions. Each treatment consisted of 30 seeds sown in individual bags. 30 bags full of black earth were arranged into square plots. This procedure was repeated three times for each treatment and for each species.

Germination behaviour of seeds

This was a study of the germination behaviour of seeds of both species during a trial of three months in duration with germination monitored daily. We conducted a daily count of the number of emerging seedlings. The data collected during these tests focused on germination, survival and germination periods.

Germination rate: The germination rate is the percentage of germinated seeds in relation to the number of seeds sown.

Germination time: The germination time is the time taken by a seed planted in earth to appear above the ground. It corresponds for this work and for each species following treatment, to the average time of seed germination during the period of three months of observation. It is obtained by the following formula:

$$Dlev(j) = \sum tlév_n / n$$

Where Dlev = average time of seed germination, expressed in days; n = total number of seeds raised; TLEV = germination time of the seed n.

Survival rate of young plants: The survival rate is the ratio between the percentage of resulting seedlings having survived after
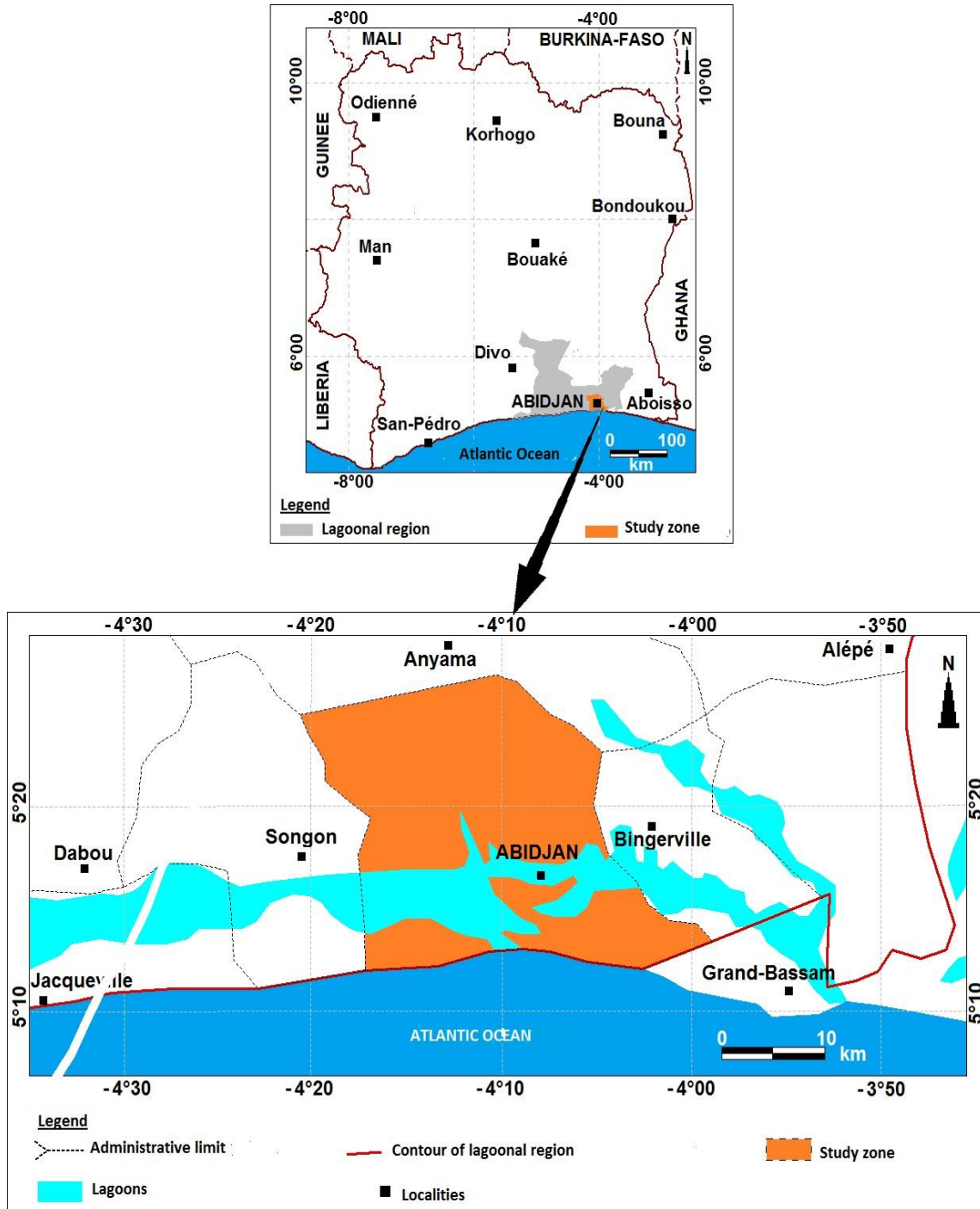
Figure 1. Geographical location of the study area.

3 months of observation and the total number of germinated seeds. It is calculated from the following formula:

$$\text{Tsurv (pc)} = \frac{S}{N} \times 100$$

S = Number of surviving plants. **N** = number of germinated seeds.

Statistical analysis of data

The analysis of variance (ANOVA) was used for exploitation of the data. The test used to compare the mean was the Dunnett test (compared to control).The analysis of variance ANOVA was used for data mining. The test used to compare the mean is the Dunnett test (procedure for comparing each experimental mean with the control mean). The average rates monitored with scarred and hardened treatments were compared to those obtained with the

Figure 2. Seeds of *Dialium guineense*.

Figure 3. Fruits and seeds of *Distemonanthus benthamianus*.

control. Treatment was different from the control when P (the associated probability) was less than 5%. The software which was used to analyse the results was XLSTAT 7.5.3 software.

RESULTS AND DISCUSSION

Morphological characteristics of seeds and fruits

Seeds and fruits of *D. guineense*

The fruits of this species are black, slightly flattened, globular, velvety domestic pods, containing a seed; however, some fruits can contain two seeds. They measure an average of 1.3 to 3.2 cm in length and 0.9 to 2.9 cm in width. The seeds are flattened and lenticular in shape with a small hollow in their centre. They are surrounded by an orange coloured mealy pulp which is sweet and tangy in flavour and edible. They are brown in colour and measure 0.8 cm in average length with a width of 0.5 to 0.7 cm, and with an average mass of 0.21 g (Figure 2).

Fruits and seeds of D. benthamianus

The fruits are domestic pods which are straw coloured, flat and papery when dry and contain from 1 to 5 seeds. They are between 8 and 12 cm in length and 2.8 to 3.5 cm in width. The seeds are oblong and elliptical in shape, and brown in colour. They weigh 0.061 g on average. They are from 0.7 to 1 cm in length and from 0.3 to 0.4 cm in width (Figure 3).

Germination behaviour

Germination period

The seeds of *D. benthamianus* germinate faster than those of *D. guineense* (Table 1). In both species, the time taken to germinate depends on the treatment method. Scarified seeds germinate faster than those of the other two lots.

Germination rate

Dialium guineense: There was a significant difference between the rates of germination of treated batches compared with the controls (Figure 4). The comparison of means (t-test) showed no significant difference between the germination rate in scarified samples and those obtained with the soaked seeds. However, germination was better in scarified seeds.

Distemonanthus benthamianus: The soaked *D. benthamianus* seeds had the best germination rate (88.90%). There is no significant difference between the average rates of germination of control seeds and scarified seeds. However, scarified seeds germinate less well than control seeds (Figure 5).

Developing seedlings from germination: Survival rate D. guineense

Table 2 presents the main results of the observations on the seedling survival rate after germination. After analysing these results, it transpires that there is no

Table 1. Average germination period according to species and treatments.

Species	Germination rate (in days)		
	Control (Te)	Soaked (T)	Scarified (S)
Dialium guineense	22,5	22,5	12,5
Distemonanthus benthamianus	11,5	9	6,5
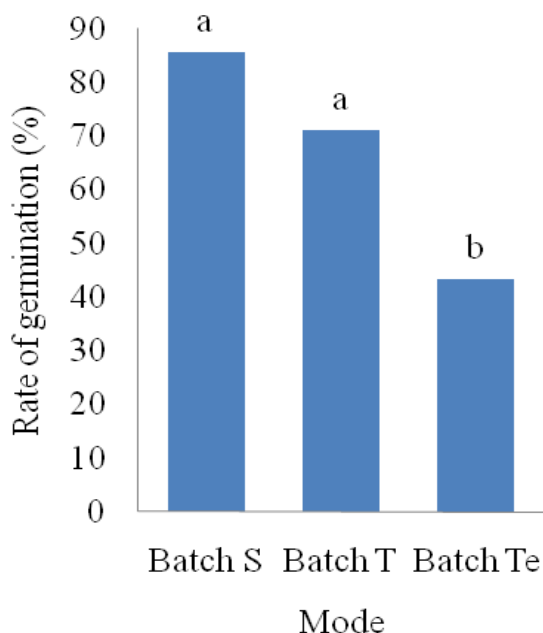
Figure 4. Consolidation of seed batches based on germination.
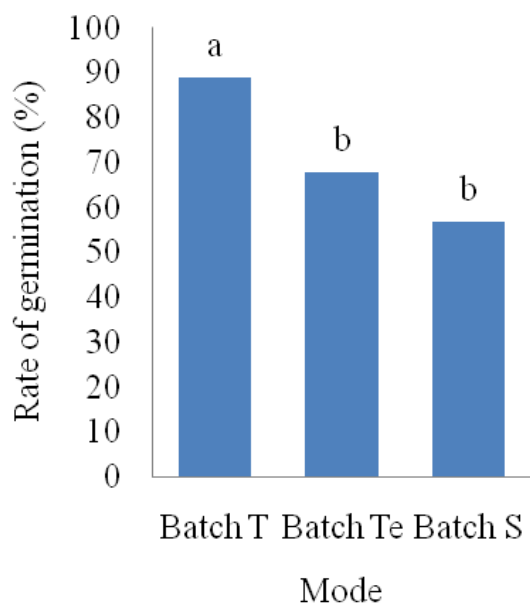
Figure 5. Consolidation of seed batches based on germination.

significant difference between the seedling survival rate of the soaked seeds and the scarified seeds. The survival rates among these are higher than those of control groups with a very significant difference.

D. benthamianus

The plants derived from soaked seeds have the best survival rates. There is no significant difference between the survival rates of scarified seeds and the survival rates of control seeds (Table 3).

DISCUSSION

Seed germination depends on the ecological characteristics of the species (Baskin and Baskin, 1998; Fenner and Thompson, 2006; Finch-Savage and Leubner-Metzger, 2006). De La Mensbruge (1966) classified species according to their germination rate, characterised as very fast when the appearance of the first emergence is obtained within two weeks after planting. Fast or medium germination is attributed to species whose emergence is between two and four weeks and is slow when it is obtained after 30 days of planting. On this basis, *D. guineense* seeds with a delay of about 22.5 days have an average speed of germination when immersed or when not receiving specific treatments. Scarification accelerates the speed, which stabilises at 12.5 days. In contrast, *D. benthamianus* is a species with rapid germination. Indeed, its germination is effective within the first two weeks after planting, with a better result when the seeds are scarified. In both cases, generally the treatment of seeds, especially scarification, accelerates the germination speed. The germination rate is considered high if it is greater than 85%, reduced when between 60 and 75%, and low if it is less than or equal to 50% (De La Mensbruge, 1966; De Koning, 1983). The results demonstrate that untreated seeds have a low germination rate in both species. It is low in *D. guineense* (43.33%) and reduced in *D. benthamianus* (67.77%). This low germination rate is common in legumes (De La Mensbruge, 1966). Similar results have been obtained with other seed species such as *Acacia spirborbis* in a study conducted in New Caledonia by Bailly (1986). For this author, this is due to the presence of a seed coat,

Table 2. Consolidation of seed batches based on survival rates.

Mode	Mean (Rate of survival in %-age)	Combinations
Batch S	85.56	A
Batch T	70.00	A
Batch Te	43.33	B

Table 3. Consolidation of seed batches based on survival rates.

Mode	Mean (Rate of survival in %-age)	Combinations
Batch T	88.889	A
Batch Te	57.778	B
Batch S	53.333	B

which is impermeable to water around the embryo. The behaviour of these species differs depending on their seed treatment conditions.

In *D. bentaniamus*, soaking improves the germination rate but scarification lowers it. In *D. guineense* germination is stronger when the seeds are scarified. The dormancy-breaking treatments have shown positive results for both species. By analysing the germination behaviour of *D. guineense* seeds with 85.55% rate of germination of scarified seed, scarification is the pre-treatment which boosted the germination rate in this species. Mechanical scarification has been successfully used to lift the seed coat dormancy of species such as *Albizia falcataria* and *Albizia lebbeck* in India (Wunder, 1966). The Mimosoideae such as *Acacia fail-fail*, *A. lebbeck*, and *Samanea saman*, have hard seeds that germinate very quickly after scarification of the seed coat (Bailly 1986). According to Kisou et al. (1983), scarification improves the seed germination rate of *Leucaena leucocephala*. Our results have shown that in *D. guineense*, species with a lignified and leathery integument, scarification improved the germination rate. This is consistent with the observations of Konate (2007), which note that the seeds with a thick, hard integumentary shell require prior scarification in order to facilitate germination. Ewedje and Tandjièkpon (2011) confirm our results with respect to ensuring that the seeds of this species should be scarified to promote germination. According to De La Mensbruge (1966), species that have good water permeability and gas exchange will germinate quickly. With a germination period reduced to 10 days, the scarified seeds of our study seem to confirm these results since scarification allows the seed to be in direct contact, or at least in part, with water and air. These results (98 seeds germinated in 9 days) were obtained by Assongba et al. (2013) in the same species. With respect to *D. benthamianus*, treatment by soaking in water at room temperature has a positive influence on the germination rate.

Thus, the best germination rate obtained (that is, 88.89%) in this case is from seeds soaked in water. Our results are consistent with those of Owusu et al. (2012), which reported a 90% germination rate in the same species in Guinea. Those authors note that soaking the seeds in water for 24 h encourages germination. In the Forest Seed Handling Guide (Kisou et al., 1983; Cousins et al., 2014), it is reported that the seed coats of some seeds are not fully permeable, hence, under these condition; the soaking of seeds in water at room temperature for 24 h promotes germination. The soaking treatment not only helps to soften the hard seed coat, but possibly leaches out inhibitory substances it could contain. The improved germination is promoted by soaking this species with its hard seed coat in cold water indicates that the hard seed coat was softened by water which has leached out any chemical inhibitors. It is noteworthy that the scarification reduced the germination time to 5 days with a low rate of germination. This poor rate was due to a deterioration of seeds that have been destroyed by pathogenic microorganisms or macroorganisms that have easier access to the seed cotyledons they house.

Conclusion

The present study on the characterisation of the behaviour of germinal seeds of *D. guineense* and *D. benthamianus*, two tree species of natural forests in Southern Ivory Coast was undertaken in preparation for their possible use in various recovery programs for the restoration of highly degraded forest cover. It is clear from our investigation that the seeds of these two species are subject to integumentary inhibition and in order to negate it and obtain a rapid germination, treatments are needed. Mechanical scarification proved better for *D. guineense* seeds. As for the *D. benthamianus* seeds, it is the process of soaking in water at room temperature that

suits them. The seedlings of these species are perfectly resistant in the environment. These results are good sign for their perfect adaptation for use as a reforestation species or improvement of fallow areas. In order to confirm this assertion, work on other aspects of their biology and ecology should be promoted, focussing on, among other things, on seedling growth rate, ability to adapt to various soil types, and more.

Conflict of Interest

The author(s) have not declared any conflict of interest.

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Full Length Research Paper

Study of potential environmental risk of trace metallic elements in mine tailings: Case of Draa Lasfar functional mine in Marrakech - Morocco

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Mining activity is one of the most important sources of heavy metals in the environment. In Marrakech region, functioning or abandoned mines represent a great hazard due to huge amounts of waste deposited in waste dumps and tailings often with high concentration of heavy metals pollution. In this study, the chemical forms of four heavy metals in mine tailings of Draa Lasfar in north-west of Marrakech city (Morocco) were studied by determining of four metals (Cd, Cu, Pb, and Zn) using standard solvent extraction and atomic absorption spectrophotometric techniques. The chemical pools of the metals indicated that the metals were distributed into six fractions with most of the metals residing in the non-residual fractions, suggesting how readily the metals are released into the environment. Results showed Cd and Cu were predominantly associated with short-term mobile fraction (F1 and F2) while Pb was largely associated with long-term mobile fraction (F3, F4 and F5) and Zn was essentially bound to Fe–Mn oxide phase (F4).

Key words: Heavy metal contamination, mining activity, sequential extraction, mine tailings, Marrakech – Morocco.

INTRODUCTION

The mining industry is an important asset for the economy of many regions across Morocco, particularly with respect to exports and employment, but also through numerous technological developments.

Active mining operations must respect governmental environmental criteria. Thus, the introduction of contaminants in the environment is limited to a level considered acceptable by the overseeing governments. Nevertheless, some abandoned / functional mine sites are not well controlled and have significant impacts on the environment (El Adnani et al., 2007).

Mining operations generate different types of
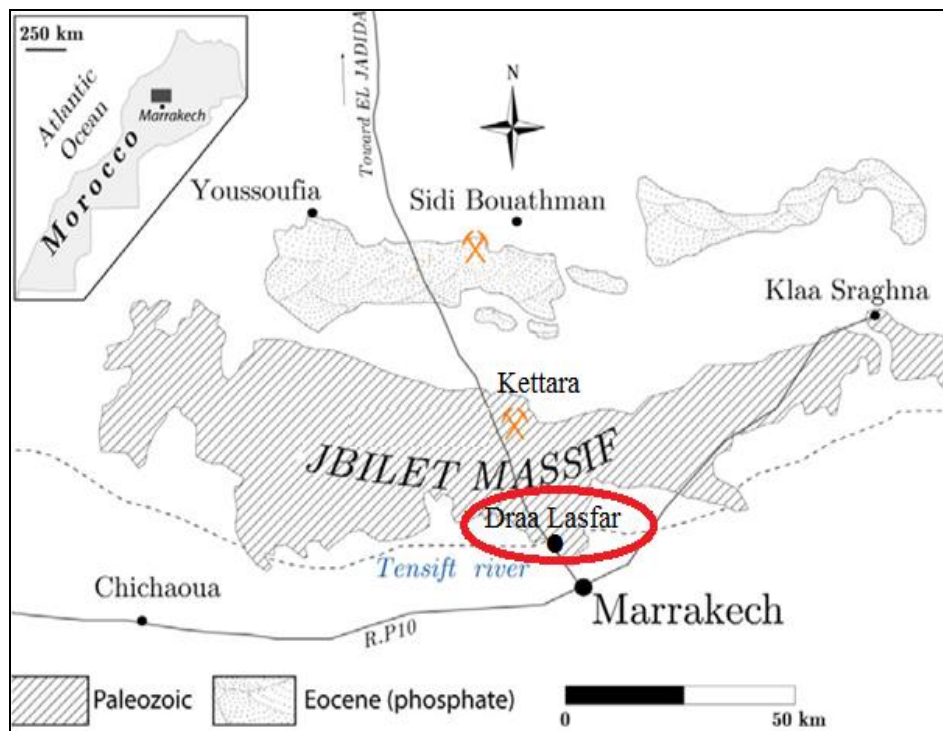
Figure 1. Location of Draa Lasfar mine.

wastes which are potentially environmentally harmful (El Gharmali et al., 2004; Aikpokpodion et al., 2013). Mining and milling/beneficiation processes (crushing, grinding, washing,) generate four major categories of waste, that is, (1) Mine waste (low-grade ore, overburden and barren rocks), (2) Tailings, (3) Dump heap leach and (4) Acid mine water (Esshaimi et al., 2012). These wastes loaded enriched in heavy metals are disposed in surrounding land and water in a more or less environmentally acceptable manner.

As a result, elevated levels of heavy metals can be found in and around disused metalliferous mines, nearby agricultural soils, food crops and stream systems, posing a potential health risk to residents in the vicinity of mining areas (Dawson and Macklin, 1998; Sahuquillo et al., 2003). The goal of this study was to determine the release of acidity and dissolved metals from the Draa Lasfar mine tailings in order to assess the impact of the functional mine residues on the biota and populations in this region.

Site description

Draa Lasfar mine, involves a pyrite deposit located 10 km northwest of Marrakech city (Figure 1) that can pose a risk for the environment due to discharge of tailings all around the mine area (Avila et al., 2012). The Draa Lasfar deposit contains 10 Mt of ore grading 5.3 wt.% Zn,

2wt.% Pb, 0.3 wt.% Cu (2007) and their orebodies consist dominantly of pyrrhotite (70 to 95 vol.% of sulphides, butcommonly up to 90 to 95 vol.% in Zn and Cu-depletedzones), with lesser sphalerite (1 to 10 vol.%), galena (0.5 to5 vol.%) and chalcopyrite (1 to 5 vol.%), and with local concentrations of deformed pyrite (2 to 3 vol.% of totalsulphides) being arsenopyrite the most common of the minor minerals (Marcoux et al., 2008).

Draa Lasfar mine is located a few hundred meters from the Tensift River, close to two rural communities (Ouled Bou Aicha and Tazakourte) of about 5790 ha of which 65% are occupied by farmland (Figure 2). The climate is Mediterranean, bordering arid and semi arid with an average annual precipitation of 231 mm (10 years). Temperatures are characterized by great daily and seasonal variation with an average value of 11.5°C in January and 36.8°C in July.

MATERIALS AND METHODS

The sampling of the tailings was carried out in 2 sampling spots (S1, S2) and (S3, S4) respectively from each tailings reservoir TR1 (155 m/100 m/13 m) and TR2 (150 m/125 m/19 m) (Figure 2) by using a specially designed cylindrical stainless steel corer. After collection, the tailing samples were carefully transferred to clean and dry self-sealing polyethylene bags and transported to laboratory. After being air-dried in paper lined propylene trays at room temperature and disaggregated with a wooden roller, all samples were sieved through <2 mm or <100 μm.

The tailings samples from each zone were thoroughly mixed and
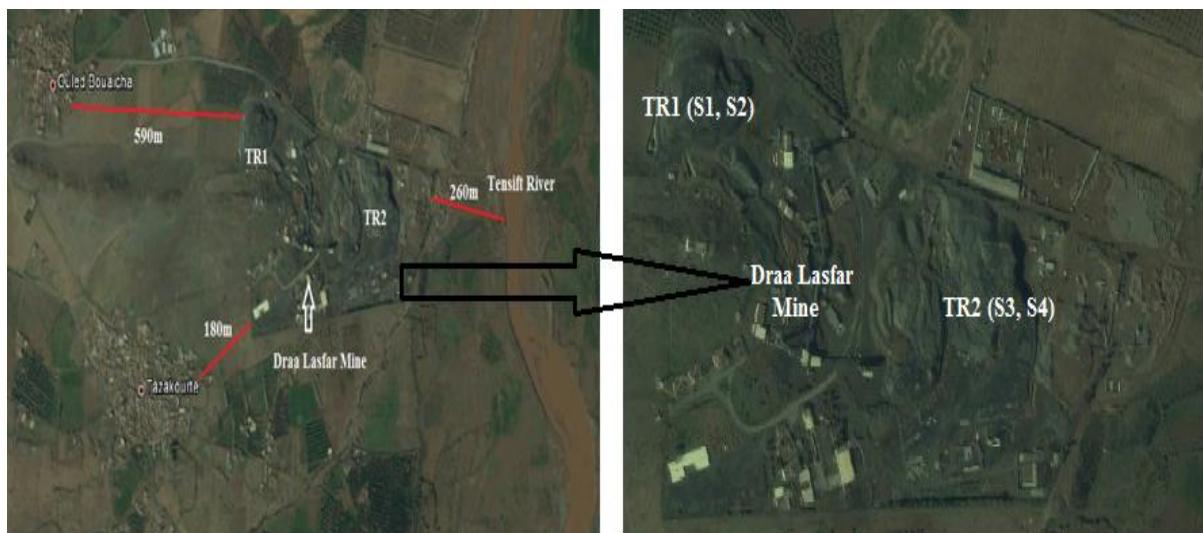
Figure 2. Panoramic sight of Draa Lasfar mine tailings.

homogenized by coning and quartering. Finally the tailings samples were stored at 4°C in tightly sealed polyethylene bags until its analysis. Due to the strong association and affinity of heavy metals with fine grained soil components, the <100 μm tailing fraction were selected for the sequential extraction and total chemical determinations (Pickering, 1986; Ramos et al., 1999; Cuong and Obbard, 2006). The <2 mm tailing fraction was used to determine the pH, electrical conductivity (EC), organic matter (OM), organic carbon (OC) and carbonate contents. The physical-chemical characterization consisted in the determination of the tailings sample pH, electrical conductivity (EC), organic matter content (OM) and the carbonate content. The soil pH was measured with the aid of pH meter while EC was done with the use of electrode meter (Badmus et al., 2014). Organic carbon content of the soil sample was estimated by following Walkey and Black method (1934). 10 g of sieved soil sample was taken into a 500 ml wide mouthed Erlenmeyer flask. To this, 10 ml of 1 N $K_2 Cr_2 O_7$ was added and swirled the flask gently to disperse the soil in the solution. 20 ml of Conc. H_2SO_4 was then added slowly followed by vigorous shaking for 1 min. Distilled water (200 ml) was added to the flask and the suspension was filtered. Few drops of o-phenanthroline indicator was then added to the filtered solution and titrated against 0.5 N $FeSO_4 H_2O$. Sample without soil was kept as a blank. The amount of organic carbon in the soil sample was calculated by using the following formula:

$$\text{Organic carbon (\%)} = \frac{(\text{milli equivalents } K_2Cr_2O_7 - \text{milli equivalents } FeSO_4) \times 0.003 \times 100}{\text{Soil weight (g)}} \times f$$

% Organic Matter = % Organic Carbon 1.724

Total heavy metal concentration was determined by atomic absorption spectroscopy after an acid digestion of samples. The methodology followed for the digestion consisted in weighting 1g dry sample and adding 3 ml HNO_3 (70%), 6 ml HCl (37%) and 3 ml HF (48%). The analyzed sample was placed in a sand bath to complete the digestion. After digestion, the sample solution was air-cooled and then diluted with deionized water.

The optimized sequential extraction procedure was applied to assess heavy metal fractionation in tailings samples (Wufem et al.,

2013). The sequential extraction was performed using five-step procedure (Fuentes et al., 2004). Additionally, a sixth step was added, which consisted of dissolving the final residue using the same digestion procedure for the total metal determination (Gleyzes et al., 2002; Avila et al., 2012).

(F1) Water Soluble: Soil sample extracted with 15 ml of deionized water for 2 h.
(F2) Exchangeable: The residue from water soluble fraction was extracted with 8 ml of 1M $MgCl_2$ (pH 7.0) for 1 h.
(F3) Carbonate-Bound: The residue from exchangeable fraction was extracted with 8 ml of 1M Ammonium acetate (adjusted to pH 5.0 with Acetic acid) for 5 h.
(F4) Fe-Mn Oxides-Bound: The residue from carbonate fraction was extracted with 0.04 M NH_2 OH. HCl in 25% (v/v) Acetic acid at 96°C with occasional agitation for 6 h.
(F5) Organic-Bound: The residue from Fe-Mn oxides bound fraction was extracted with 3 ml of 0.02M HNO_3 and 5 ml of 30% H_2O_2 (adjusted to pH 2 with HNO_3) was added and the mixture heated to 85°C for 3 hours, with occasional agitation. A second 3 ml aliquot of 30% H_2O_2 (pH 2 with HNO_3) was added and the mixture heated again to 85°C for 3 h with intermittent agitation.
After cooling, 5 ml of 3.2 M NH_4 acetate in 20% (v/v) HNO_3 was added and the samples was made up to 20 ml with deionized water and agitated continuously for 30 min.
(F6) Residual: The residue from organic fraction after drying was digested in a conical flask with 10 ml of 7M HNO_3 on a hot plate for 6 h. After evaporation, 1 ml of 2 M HNO_3 was added and the residue after dissolution was diluted to 10 ml. The residue was washed with 10 ml of deionized water. After each successive extraction separation was done by centrifuging at 4000 rpm for 30 min. The supernatants were filtered and analyzed for heavy metals. All chemicals used were of reagent grade and pure deionized water was used throughout the experimentation. All plastic were soaked in 10% HNO_3.

RESULTS AND DISCUSSION

The results obtained for the tailing grain-size, pH, EC, OM, OC and $CaCO_3$ content measurements

Table 1. Mean values (%) of the grain-size analysis and geochemical parameters of different tailing samples from Draa Lasfar mine.

Variables	S1	S2	S3	S4
clay	25.7 ± 3.8	27.7 ± 4.5	29.1 ± 3.1	27.4 ± 3.5
Fine silt	18.1 ± 4.7	14.7 ± 3.7	13.5 ± 2.7	15.6 ± 2.7
Coarse silt	9.2 ± 3.5	10.5 ± 1.8	13.8 ± 1.5	13.1 ± 3.2
Fine sand	24.7 ± 3.7	21.4 ± 3.4	19.7 ± 3.1	19.2 ± 4.7
Coarse sand	22.5 ± 4.1	25.7 ± 5.8	23.9 ± 2.8	24.1 ± 8.4
pH	7.5 ± 0.5	7.4 ± 0.5	7.6 ± 0.5	7.2 ± 0.6
E.C (μS.cm^{-1})	1782.7 ± 421.3	1549.5 ± 348.5	1257.9 ± 264.2	1865.4 ± 421.7
OM (%)	2.7 ± 1.0	3.5 ± 0.5	3.2 ± 1.7	2.3 ± 0.8
OC (%)	1.6 ± 0.6	2.0 ± 0.4	1.9 ± 1.0	1.3 ± 0.3
$CaCO_3$ (mg.g^{-1})	181.5 ± 17.1	196.4 ± 32.1	162.1 ± 21.4	168.2 ± 22.5
SO_4^{2-}	3.7 ± 0.6	3.4 ± 0.4	3.9 ± 0.6	3.7 ± 0.6

Table 2. Mean total concentrations of heavy metals of different tailing samples of Draa Lasfar mine.

Variables	S1	S2	S3	S4
Cd (mg.kg^{-1})	143.1 ± 12.7	127.8 ± 13.4	157.2 ± 8.8	96.7 ± 9.4
Cu (mg.kg^{-1})	871.9 ± 25.8	971.2 ± 42.4	969.1 ± 38.7	950.9 ± 35.2
Pb (mg.kg^{-1})	3381.7 ± 117.1	3144.5 ± 34.7	2942.7 ± 37.7	3108.8 ± 41.8
Zn (mg.kg^{-1})	2478.5 ± 124.2	2123.7 ± 95.7	2268.1 ± 67.8	2398.4 ± 78.5

corresponding to the old Draa Lasfar tailing mine are presented in Table 1. These results show that OC of different tailing samples depends on the sand composition of tailings. The low OC values of different soils is related with the low rate of degradation by microbial-mediated processes due to the heavy metal contamination. These are also probably related to the poor absorbability of organics on negatively charged quartz (Aysha et al., 2015) which predominate in solid tailings of Draa Lasfar mine. In addition, the constant flushing activity by rain can support the low percentage of OC in these soils.

Results show also that Draa Lasfar mine tailing samples have neutral to alkaline pH for the majority of the samples. Neutral and alkaline pH in mining residues could be attributed to the presence of carbonates with a high concentration ranging from 162.1 to 196.4 mg g^{-1} for tailings, due primarily to the mixture of these mining residues with soil very rich in carbonates. The EC measurements revealed that all tailings samples presented high values ranging from 1257,9 to 1865.4 μS cm^{-1}. Results showed that the most alkaline tailings samples have the lower EC values. This correlation between high pH and low EC value can be explained by the presence of low amounts of sulfur ions that causes a decrease of the EC. Regarding the carbonate content, it can be stated that tailings with a pH of 7.1 and higher generally have high calcium carbonate content. Total element concentrations in the investigated soils varied in

a narrow range of values (Table 2). In this sense, alkaline tailing samples together with high amounts of OM and the presence of carbonates increase the retention of heavy metals in these tailing samples.

However, these values do not provide sufficient information of its potential hazardous effects on environment because the mobility and eco-toxicity of heavy metals depend strongly on their specific chemical forms or binding. Heavy metals may be distributed among many components of the tailings and may be associated with them in different ways (Aikpokpodion et al., 2013) and the determination of the heavy metals fractionation is useful in order to assess toxic effects. The chemical form of heavy metals in tailing components is of great significance in determining the potential bioavailability and translocation of the metals to other environmental compartments like water, soil, plant and microorganisms when physicochemical conditions are favorable (Cuong and Obbard, 2006).

Overall, Cd is well distributed among the different fractions in all tailing samples (Figure 3) and seems to be very available with more than 34.1 to 42.7% of this metal bound to the short-term mobile fraction F1 and F2 respectively. The long-term mobile fraction (F3, F4 and F5) homes more than 34.5 to 38.5% of Cd. The immobilized fraction (F6) traps about 18.3 to 24.5%.

Copper in tailings (Figure 4) is essentially bound to soluble (F1) and exchangeable fractions (F2) with more than 34.7 to 37% of the total metal content. Copper is
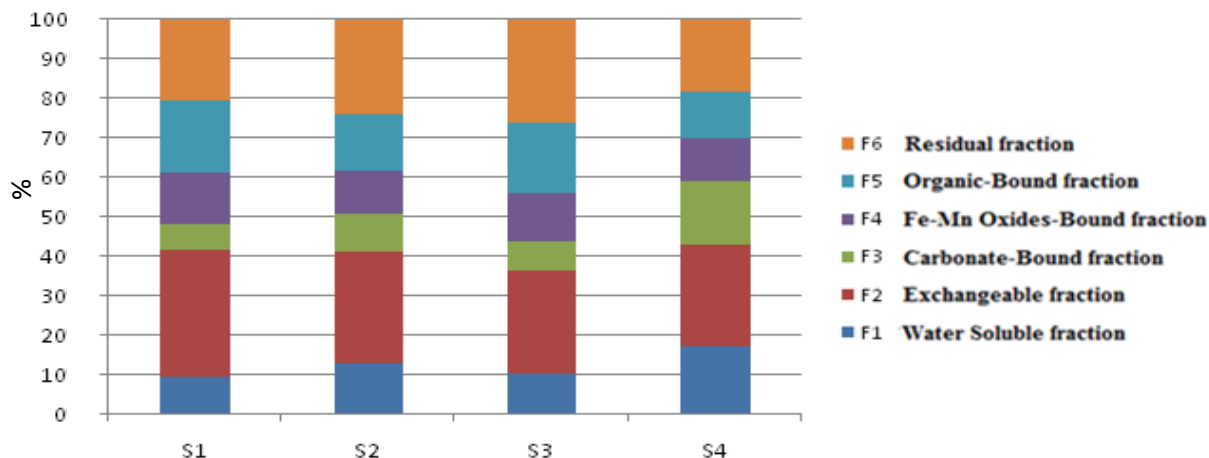
Figure 3. Relative distribution of Cd among six fractions (F1-F6) obtained by chemical extraction of studied tailing samples.
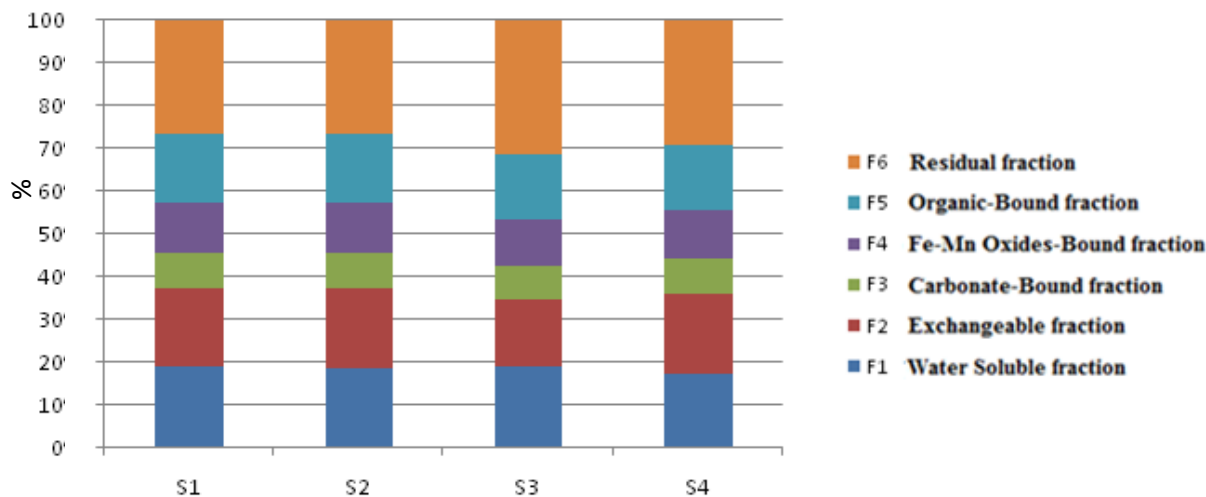
Figure 4. Relative distribution of Cu among six fractions (F1-F6) obtained by chemical extraction of studied tailing samples.

also widely present (26.5 to 31.5%) in the residual fraction (F6). The oxidizable (F3), acid-soluble (F4) and reducible factions (F5) are represented by small percentages in these tailings, representing respectively about 7.9 to 8.5, 10.9 to 11.7 and 14.8 to 16%.

The high percentage of Cu in the residue is likely due to the fact that Cu is easily chemisorbed on or incorporated in clay minerals (Pickering, 1989). The significant Cu concentrations in organic phase can be justify by Cu affinity with OM, thus it can be hypothesized that Cu is bound to labile organic matter such as lipids, proteins, and carbohydrates. Lead distribution (Figure 5) in tailings is characterized by a strong dominance of this metal bound to long-term mobile fraction (F3, F4 and F5), with more than 50.4 to 60% of the total metal. The short-

term mobile fraction (F1 and F2) represents about 18 to 21.5%. The remaining fraction (F6) traps about 19 to 28% of the total metal.

The relatively high percentage of Pb in reducible phase is in agreement with the known ability of amorphous Fe–Mn oxides to scavenge Pb from solution (Yusuf, 2007). Thus, a high Pb percentage in the reducible fraction is a hazard for the aquatic environment because Fe and Mn species can be reduced into the porewaters during early diagenesis by microbially mediated redox reactions (Oluwatosin et al., 2008). Dissolution will also release Pb associated with oxide phases to the porewater possibly to the overlying water column and to benthic biota (Kabala and Singh, 2001). The major sources of Pb are from intensive human activities, including agriculture in the
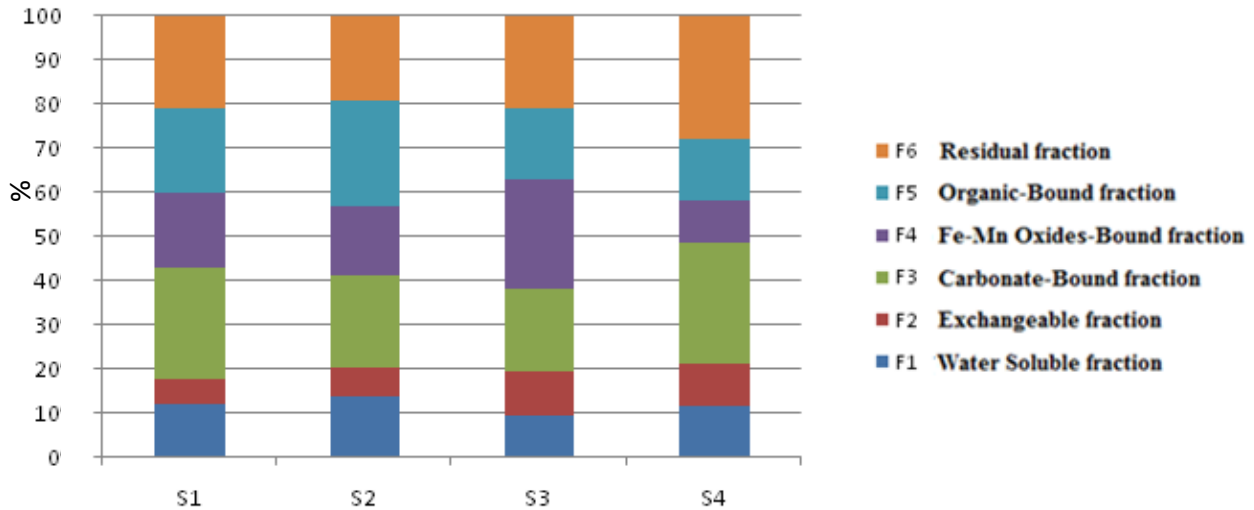
Figure 5. Relative distribution of Pb among six fractions (F1-F6) obtained by chemical extraction of studied tailing samples.
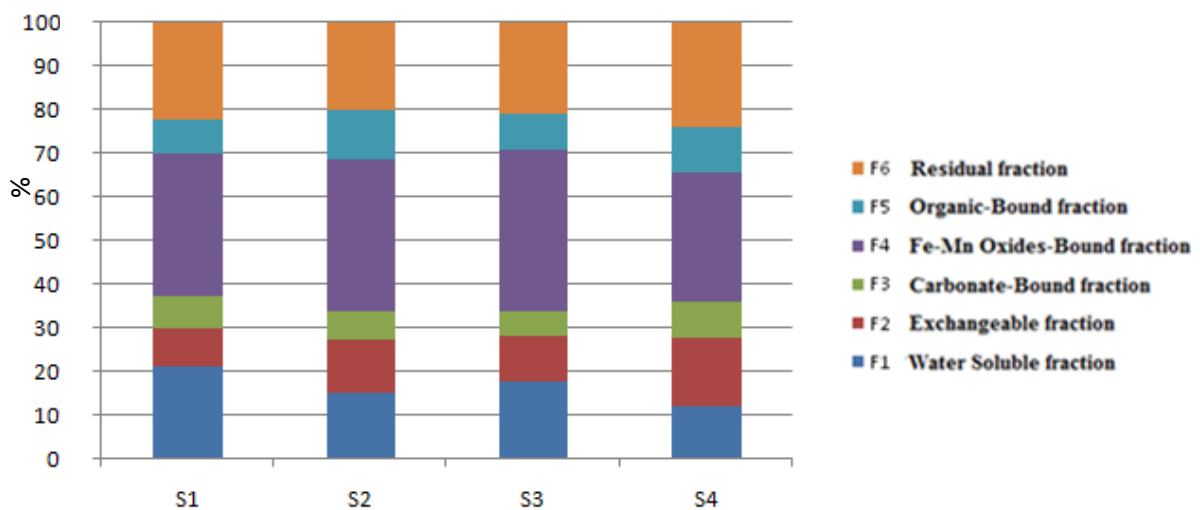
Figure 6. Relative distribution of Zn among six fractions (F1-F6) obtained by chemical extraction of studied tailing samples.

drainage basin (Rodriguez et al., 2009). In addition, a substantial contribution from the factories located in the upstream of the Tensift river dealing with Pb play a vital role in soils contamination by heavy metals as referred by Sarkar et al. (2007).

Zinc in tailings (Figure 6) is essentially bound to Fe–Mn oxide phase (F4) with more than 32 to 39% of the Zn content. This result can be justified by the high stability of Zn on oxides. Iron oxides adsorb considerable amounts of Zn and these oxides may also occlude Zn in the lattice structures (Banerjee, 2003). Zinc is also widely present (21 to 26%) in the residual fraction (F6). The water soluble (F1) and exchangeable fractions (F2) are represented by relatively small percentages in these

tailings (about 13 to 22% and 9 to 14% respectively).

Conclusions

Environmental pollution by heavy metals originated from functional mines can become a very important source of contamination both in soil and water. Therefore, the characterization of chemical and physical properties tailings is important to assess the risk of potential environmental mobility of toxic trace metals that are contained in this kind of waste. Taking into consideration the high mobility and potential bioavailability of heavy metals in this fraction and their total concentration, it can

be concluded that Draa Lasfar mine tailings could have potentially hazardous effects on the environment. Cadmium and copper were predominantly associated with short-term mobile fraction (F1 and F2) while lead was largely associated with long-term mobile fraction (F3, F4 and F5) and Zn was essentially bound to Fe–Mn oxide phase (F4).

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

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Full Length Research Paper

Wood ash as corrective of soil pH and as fertilizer in ornamental sunflower cultivation

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Wood ash, as having required nutrients by plants, can be an alternative in fertilization and soil acidity correction in agricultural crops. This study aimed to evaluate the development of the sunflower ornamental cultivar (*Helianthus annuus* L.) Sunflower F1 Sunbright Supreme when subjected to wood ash fertilization into of Cerrado Oxisol. The experiment was conducted in a greenhouse, in the municipality of Rondonópolis-MT, with six treatments (0, 4, 8, 12, 16 and 20 g dm^{-3} of wood ash) and six repetitions. The soil sample was collected from 0 to 0.20 m depth. Data were collected at 30, 37, 44 and 51 days after sowing on plant height, stem diameter, leaf number and chlorophyll index. Chapter diameter and height and disc diameter variables were assessed at 44 and 51 days after sowing, respectively. Data were subjected to analysis of variance and regression test at 5% probability through the SISVAR statistical program. Wood ash enhanced ornamental sunflower plant growth at doses between 12 and 16 g dm^{-3}, satisfying the commercial standards required by the flower market.

Key words: Oxisol, agriculture solid waste, *Helianthus annuus* L.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is a plant from the southwestern part of Mexico. This species was introduced in Europe in the fourteenth century as cultivated plant, and after in America in the nineteenth century. Its utilization as an ornamental plant is intended for flowers production, a practice that has grown in the Central-South region of Brazil (Neves et al., 2005).

Floriculture practice generally comprises ornamental plant cultivation, as well as cuts cultivation and large-sized tree seedling production (Kiyuna et al., 2004). One of the options that add most value to the industry is potted plant sale, such as the ornamental sunflower, which has potential for confined spaces cultivation and has short cycle, without handling difficulties (Braga, 2009). As Cerrado soils usually have high acidity and natural fertility limitations, especially in relation to low pH, high exchangeable aluminum content and low nutrient content, especially phosphorus, it is necessary to correct these growth limits in order to have satisfactory productions (Santana, 2009). As an alternative to reduce fertilizer costs, agro-industrial by-products, use were found, such as wood ash, as it consequently reduces the

Table 1. Soil chemical and particle size properties.

pH		P	K	Ca+Mg	Ca	Mg	Al	H	M.O	Sand	Silt	Clay
H_2O	$CaCl_2$	mg dm^{-3}		-------------$cmol_c$ dm^{-3}-------------					g dm^{-3}	----------g Kg^{-1}-----------		
4.7	4.0	1.7	24	0.4	0.2	0.2	0.8	4.4	20.6	507	116	377

Table 2. Wood ash chemical content.

$pH(H_2O)$	N	P_2O_5 Total	K_2O	Zn	Cu	Mn	B	Ca	S	Fe
					-----$CNA+H_2O$-----					
					-----g Kg^{-1}-----					
11.8	2.5	48.5	16.6	0.13	0.0	0.5	0.2	37.5	2.8	15.3

need for commercial fertilizer use, thereby contributing to soil acidification reduction and increasing calcium supply (Zimmermann and Frey, 2002; Souza et al., 2013).

Waste use, such as wood ash from power generation, industrial boilers burning, is a standard practice that helps minimize environmental impacts and mineral fertilizer acquisition and application costs (Bonfim-Silva et al., 2011a). Wood ash, when applied to the soil, reduces acidity and increases base saturation, which is useful as a corrective of soil pH (Maeda et al., 2008). Furthermore, when used as a fertilizer, wood ash that contains phosphorus, potassium, calcium and magnesium minerals, in addition to other nutrients that influence the development and favor plant cultivation, resulting in significant productivity gains (Moro and Gonçalves, 1995; Bonfim-Silva et al., 2014; Santos et al., 2014).

In this context, having a great potential use in agricultural, wood ash plays an important role in fertilization and management practices of low fertility soils (Bonfim-Silva et al., 2013) in order to reduce chemical fertilizers cost in flowers production (Pereira, 2014). Thus, the aim of the present study was to assess the influence of wood ash fertilization on ornamental sunflower plant development in Cerrado Oxisol.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse from April to July 2014 in the Federal University of Mato Grosso, Rondonópolis Campus, located at 16°28'15" South latitude, 54°38'08" west longitude and 290 meters altitude. The experimental design was randomized blocks, with six wood ash doses (0, 4, 8, 12, 16 and 20 g dm^{-3}) and six repetitions. The soil used in the experiment, Oxisol (Embrapa, 2013), was collected in Cerrado area vegetation in the 0 to 0.20 m depth. The soil was then sieved with a 4 mm mesh and used to fill the pots.

A sub-sample of this soil was sieved on a grid of 4 mm and the particle size and chemical content were determined according to the EMBRAPA (1997) (Table 1). The wood ash was obtained from eucalyptus wood (*Eucalyptus* spp.) used in food industry burning boiler (Table 2) and was characterized as fertilizer according to Darolt et al. (1993).

The wood ash was mixed with the soil and all was incubated for 30 days in plastic pots of 1.3 dm^3 volume and kept at 70% of soil maximum water holding capacity, according to the method described in Bonfim-Silva et al. (2011b). 20 mg dm^{-3} of nitrogen at sowing and 80 mg dm^{-3} of nitrogen 20 days after sowing was applied as urea solution. Three seeds of Sunflower F1 Sunbright Supreme cultivar ornamental sunflower (*H. annuus* L.) were sown per pot, and thinning was carried out 15 days after sowing, leaving one plant per pot.

Data were collected on plant height, stem diameter, leaf number and chlorophyll index at 30, 37, 44 and 51 days after sowing. For the chapter diameter, and height and diameter of the disc, two growth assessments were made at 44 and 51 days after sowing, respectively. The soil pH (H_2O) was determined after 30 days of incubation, with the removal of 1cm^3 of soil from each pot. Each sample was stored in plastic glasses of 100 ml. 25 ml of water was added, and it was left for 60 min, afterwards, the reading of each sample was performed.

Plant height (cm) was measured using a ruler graduated in centimeters, from soil surface to the plant apex distance, or up to inflorescence insertion point as present in Figure 1A. The stem diameter (cm) was measured with a digital caliper in the middle third of the main stem (Figure 1B). The determination of leaf number per plant was done by counting all leaves produced by the plants during the experiment.

Root, stem, leaf and chapter dry matter (g plant^{-1}) were determined at 51 days after sowing. The shoot was separated into stem, leaves and chapter, and roots were washed under running water. After dry matter weighing, parts were separated and placed in paper bags and taken to an oven with forced air circulation at 65°C, up to constant weight. When achieving constant mass, shoot and roots were weighed in analytical balance, in order to determine the dry weight. Chapter height (cm) was measured with the aid of a ruler graduated in centimeters, from soil surface to the plant apex distance (Figure 2B).

Disc and chapter diameter (cm) were measured with a ruler graduated in centimeters. Floral chapter diameter was obtained by measuring the inflorescences diameter when they were fully formed. Disc diameter was measured when the chapter was completely formed (Figure 2A). Leaves Falker's chlorophyll index (chlorophyll content indirect determination) was assessed using an electronic meter (ClorofiLOG - CFL 1030®). The measurement was made on three plant middle third leaves (Figure 3).

Data were subjected to analysis of variance and regression test at 5% probability with the SISVAR statistical program (Ferreira, 2011). The wood ash doses that provided the maximum values for the variables that adjusted the quadratic regression model were
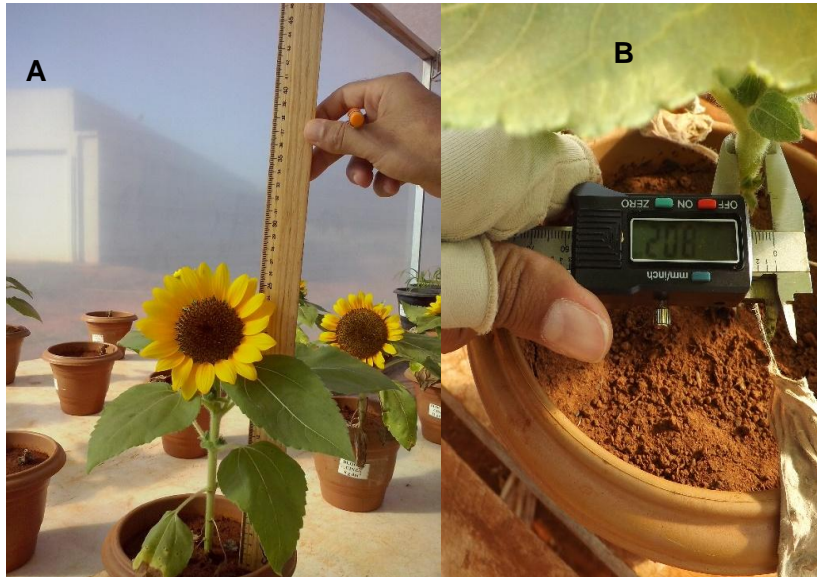
Figure 1. Ornamental sunflower plant height (A) and stem diameter measurement (B).
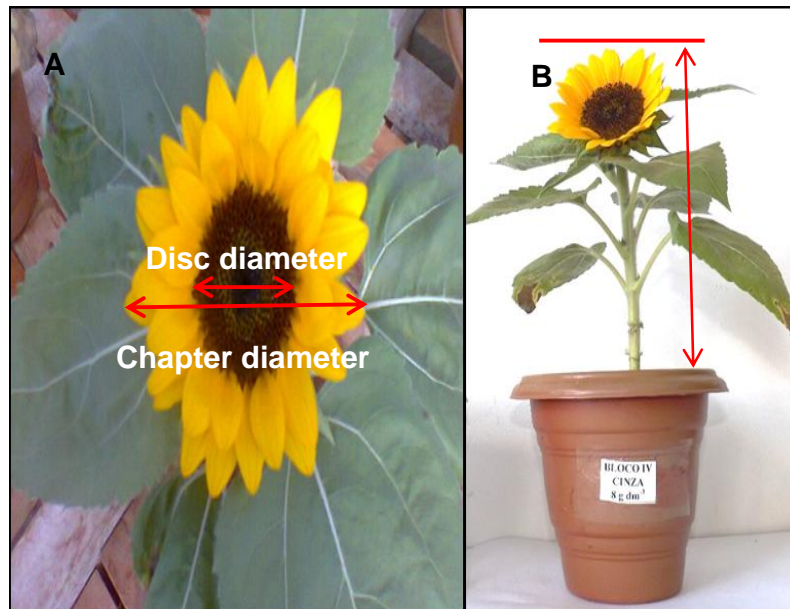
Figure 2. Ornamental sunflower disc and chapter diameter (A) and chapter height (B).

determined by the first derivative of the equations.

RESULTS AND DISCUSSION

The assessments of the Sunflower Chlorophyll index did not show a significant effect of wood ash doses.

This could be occurred because all treatments received the same nitrogen fertilization (100 mg dm^{-3}). For soil pH, plant height, stem diameter, leaf number, chapter, leaf and stem dry matter and chapter height and diameter, there was a significant effect by adjusting linear and quadratic regression models (Table 3).

Soil pH after 30 days of incubation with wood ash was

Figure 3. Ornamental sunflower plants indirect chlorophyll content readings with a chlorophyll meter.

Table 3. Summary of ANOVA for the parameters: plant height and chapter diameter , stem diameter, chlorophyll index , chapter dry mass , stem and disc , leaf and root , number of leaves and soil pH.

Parameter	Evaluations	F_t	Cofficient of variation (%)
Chlorophil index	1°	0.3829^{ns}	8.91
	2°	0.4476^{ns}	5.46
	3°	0.8761^{ns}	8.01
	4°	0.2392^{ns}	7.89
Soil pH	1°	0.0000^{***}	2.56
Plant height	1°	0.0000^{***}	19.66
	2°	0.0000^{***}	12.25
	3°	0.0000^{***}	9.89
	4°	0.0000^{***}	10.42
Stem diameter	1°	0.0000^{***}	8.80
	2°	0.0000^{***}	17.79
	3°	0.0000^{***}	13.34
	4°	0.0000^{***}	7.73
Number of leaves	1°	0.0000^{***}	10.99
	2°	0.0000^{***}	10.84
	3°	0.0000^{***}	10.61
	4°	0.0000^{***}	10.61
Chapter dry mass	4°	0.0000^{***}	19.53
Leaves dry mass	4°	0.0000^{***}	17.97
Stem dry mass	4°	0.0000^{***}	23.67
Root dry mass	4°	0.0000^{***}	53.90
Chapter height	3°	0.0078^{**}	8.22
	4°	0.0020^{**}	8.92
Chapter diameter	3°	0.0044^{**}	18.86

Table 3. Contd.

	4°	0.0355*	11.28
Disc diameter	3°	0.0017**	17,20
	4°	0.0015**	13.71

[ns]non significant, ***, **, * significant at 0.1, 1 and 5% of probability, respectively, by the F test.
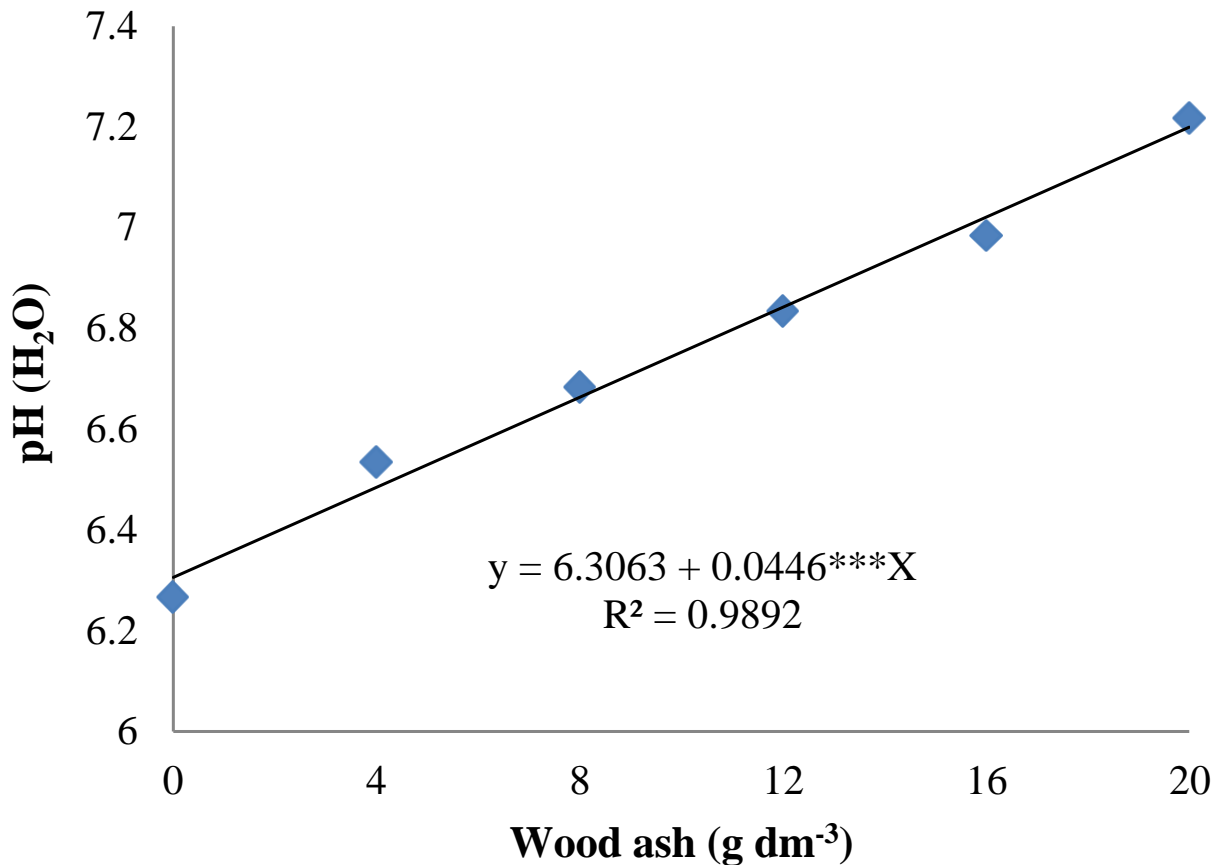
Figure 4. Soil pH after 30 days of incubation as function of wood ash doses in CerradoOxisol. *** 0.1% significance.

$$y = 6.3063 + 0.0446***X$$
$$R^2 = 0.9892$$

set to the linear regression model, where the highest pH value (7.2) was observed in the 20 g dm^{-3} wood ash dose (Figure 4). Comparing the highest pH found with the value obtained in the wood ash fertilization absence, there was an increase of 13.8%.

The pH increase increased with the wood ash doses may be related to soil acidity neutralizing capacity that wood ash has (Osaki and Darolt, 1991). Wood ash oxides, hydroxides and carbonates of Ca and Mg and basic cation significant amounts, such as calcium, magnesium and potassium reduce soil acidity and increase soil pH, improving its fertility (Haraldsen et al., 2011; Norstrom et al., 2012). Similar effect to that of the present study was observed by Ferreira et al. (2012)

when wood ash was used as fertilizer in Latossol. In fact, these authors find that soil content of H + Al significantly decreased with wood ash rate increase. At 30 days after sowing, it was observed that the highest plant height (14.15 cm) was achieved with a 13.01 g dm^{-3} wood ash dose, with an increase of 88.9% compared to the treatment that had not received wood ash fertilization (Figure 5). During the second evaluation, at 37 days after sowing, a plant height increase of 75.5% was observed, with maximum height (19.79 cm) being observed in the 12.94 g dm^{-3} wood ash dose (Figure 6).

At 44 days after sowing, 12.07 g dm^{-3} of wood ash allowed the highest plant height (18.02 cm), giving an increase of 72.1%. Similarly, at 51 days after sowing, the

A

$y = 3.925595 + 1.571890{***}X - 0.0604{***}X^2$
$R^2 = 0.9103$

B

$y = 4.843929 + 2.309479{***}X - 0.0892230{***}X^2$
$R^2 = 0.8213$

C

$y = 5.083333 + 2.173214{***}X - 0.090030{***}X^2$
$R^2 = 0.6479$

D

$y = 4.975 + 2.238720{***}X - 0.092299{***}X^2$
$R^2 = 0.6366$
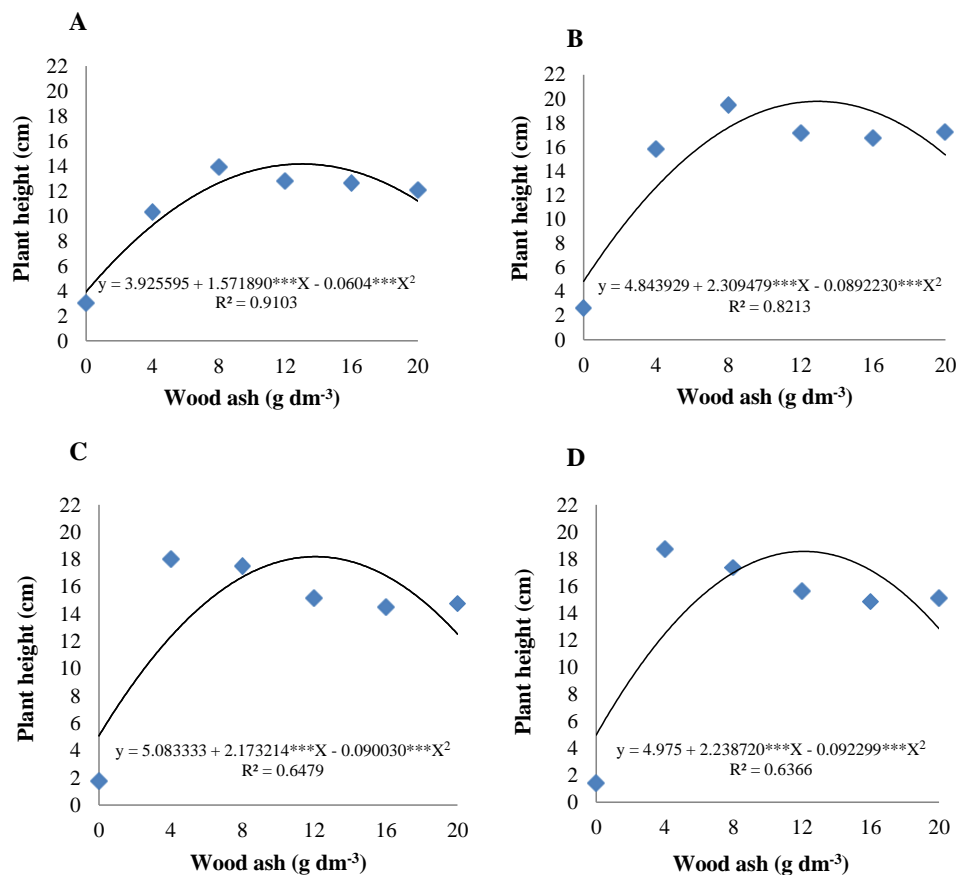
Figure 5. Ornamental sunflower plant height at 30 (A), 37 (B), 44 (C) and 51 (D) days after sowing, depending on wood ash doses. *** 0.1% significance.
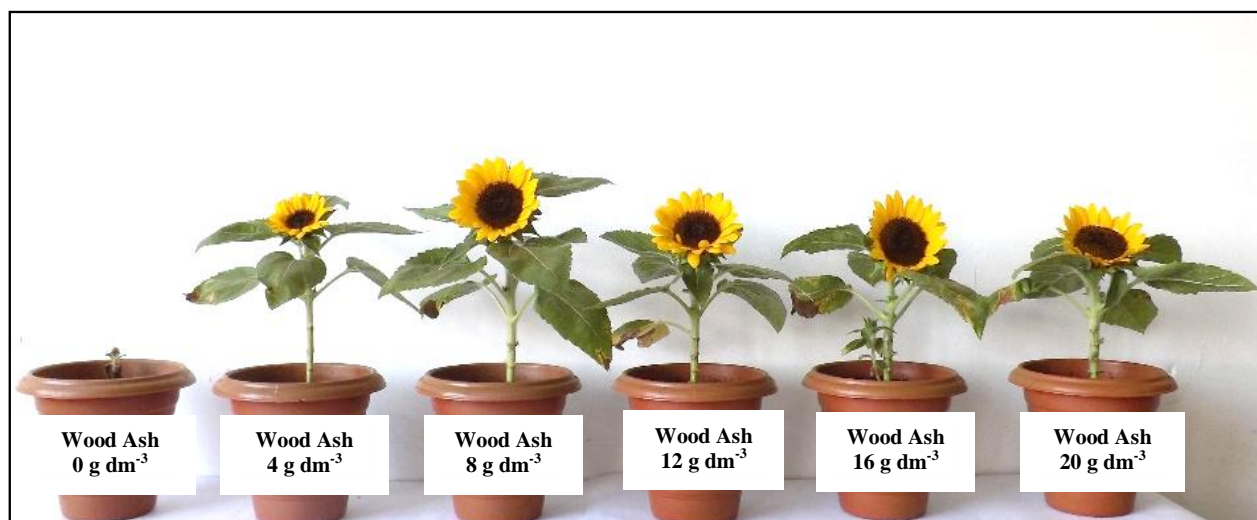
Figure 6. Growth curve of ornamental sunflower as influenced by wood ash doses at 37 days after sowing.

highest height of 18.55 cm was obtained with 12.13 g dm^{-3} wood ash dose, giving an increase of 73% (Figure 5).

The highest values for the height (18.55 and 18.20 cm) were obtained with 12.07 and 12.13 g dm^{-3} of wood ash,

A

$y = 2.586131 + 0.715106^{***}X - 0.02397^{***}X^2$
$R^2 = 0.9419$

B

$y = 3.406905 + 0.721113^{**}X - 0.022924^{***}X^2$
$R^2 = 0.9610$

C

$y = 2.675298 + 0.968847^{***}X - 0.032130^{***}X^2$
$R^2 = 0.9747$

D

$y = 2.809405 + 0.860461^{***}X - 0.027556^{***}X^2$
$R^2 = 0.9375$
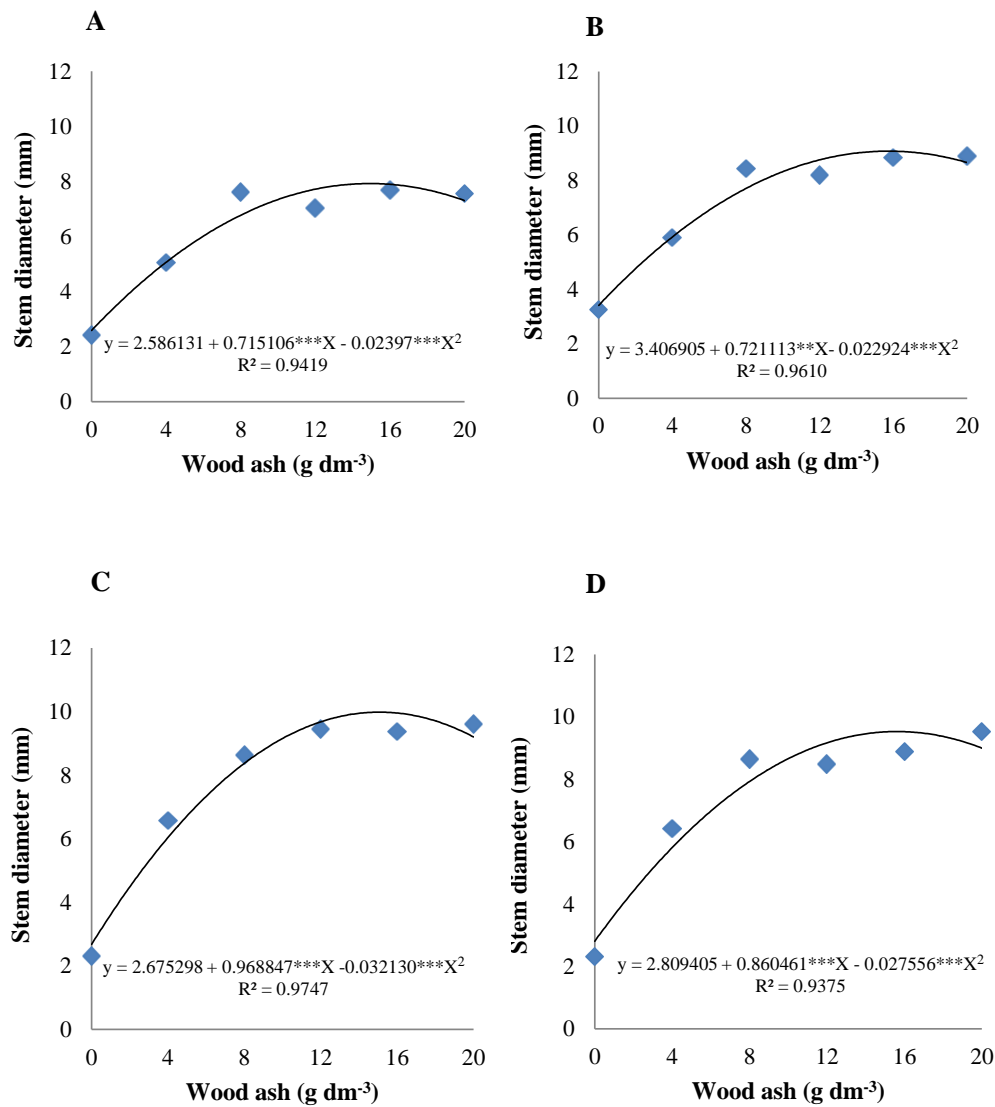
Figure 7. Ornamental sunflower stem diameter at 30 (A), 37 (B), 44 (C) and 51 (D) days after sowing as influenced by wood ash doses. *** = 0.1% significance.

which were observed in 44 and 51 days after sowing, respectively (Figure 5). However, curves adjusted to plant height in the third (Figure 5C) and fourth (Figure 5D) assessments had lower determination coefficients (R^2) when compared with the first two assessments.

It was observed during the experiment that plants fertilized with the wood ash dose of 8 g dm^{-3} showed, on average, the highest heights, until the 37th day after sowing. From the third assessment, plants fertilized with 4 g dm^{-3} of wood ash had higher plant height than others. This occurred was due to long vegetative stage observed in plants subjected to 4 g dm^{-3} of wood ash when compared to other plants that have reached the beginning of the reproductive stage (chapter formation) at around 30 days after sowing. According to Castiglioni et al. (1997), when reaching the reproductive stage, sunflowers have already grown around 95% of their total.

Setting a standard height for the ornamental sunflower potted in the flower market is a difficult task, and is a subjective characteristic that depend on consumer preference. However, are found in the flower market, plants with an average of 25 cm (Neves et al., 2005). In the present study, the plants showed sizes that are more compact what is feasible and facilitates transport, without damaging the plants. In addition, the appropriate transport does not compromise the quality of flowers, where the aesthetics of the product determines the market value of the plant (Mcmahon and Kelly, 1999; Curti et al., 2012).

As for stem diameter, for the four assessments, it can be observed adjust of the data for the quadratic regression model (Figure 7). In the first assessment, the highest diameter (7.92 mm) was observed in the 14.92 g dm^{-3} wood ash dose, and in the second, it was found that

A

$$y = 7.732143 + 0.883185^{***}X - 0.028088^{***}X^2$$
$$R^2 = 0.9423$$

B

$$y = 9.529762 + 0.726339^{***}X - 0.025484^{***}X^2$$
$$R^2 = 0.9247$$

C

$$y = 9.267857 + 0.708482^{***}X - 0.023996^{***}X^2$$
$$R^2 = 0.9794$$

D

$$y = 9.2679 + 0.7085^{***}X - 0.024^{***}X^2$$
$$R^2 = 0.9794$$
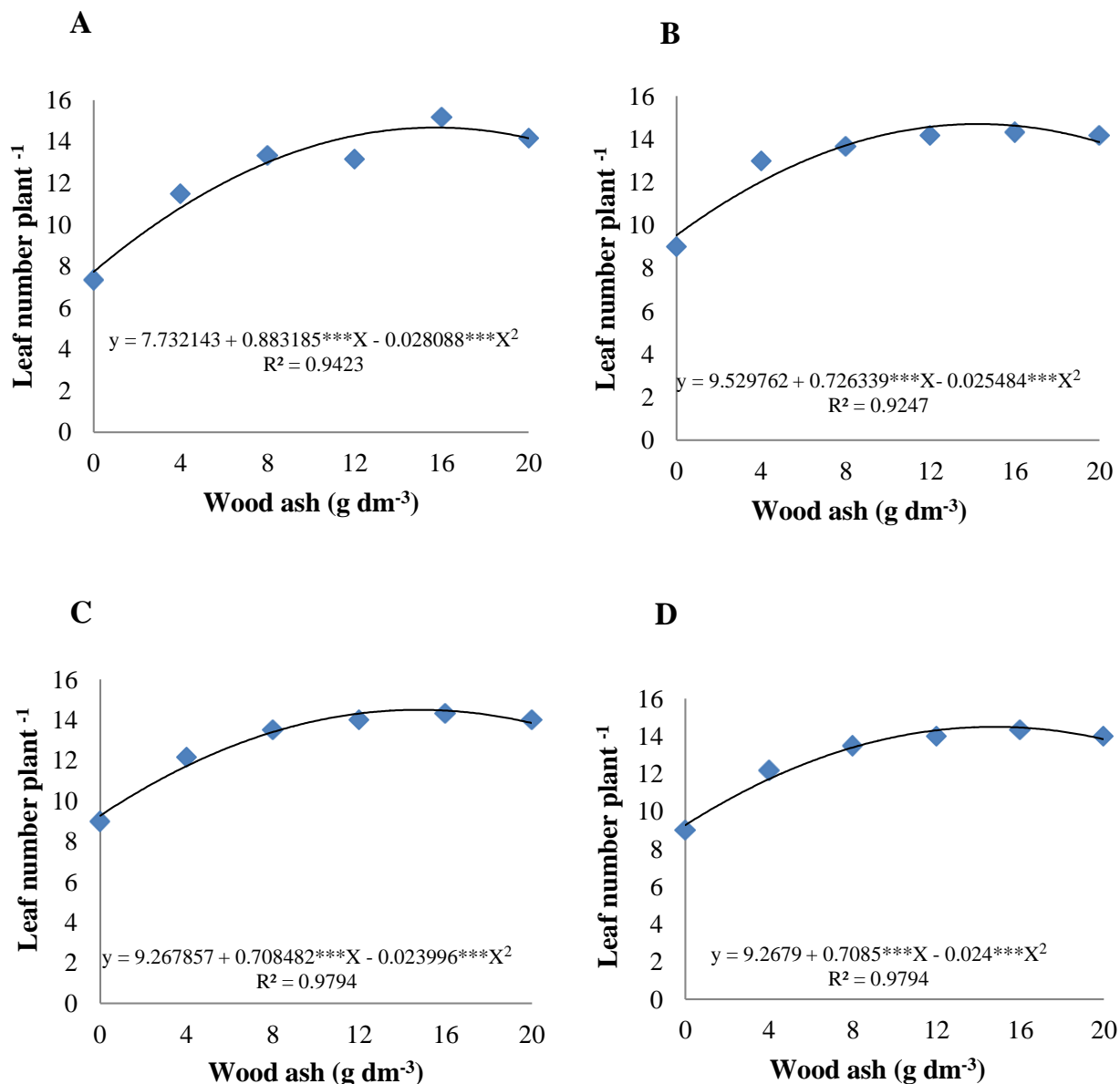
Figure 8. Leaf number at 30 (A), 37 (B), 44 (C) and 51 (D) days after sowing influenced by wood ash doses. *** 0.1% significance.

15.72 g dm^{-3} wood ash dose provided higher plant stem diameter of 9.08 mm.

In the third assessment, it was observed that the 17.08 g dm^{-3} wood ash dose favored plants to develop stem with higher diameters (9.85 mm). According to the fourth assessment results, it appears that the largest diameter (9.53 mm) was found in the 15.62 g dm^{-3} wood ash dose. Stem diameter increases measured at 30, 37, 44 and 51 days after sowing were 67.3, 62.4, 72.8 and 70.5%, respectively..

This study results corroborate the observation of Braga (2009) on ornamental sunflower development in relation to nitrogen doses, at 40 days after sowing, that noted that

the largest stem diameter was 9.70 mm. According to Sabach (2008), stem diameter lower than 6 mm is not favorable, since stems with lower diameter are flexible, compromising inflorescence sustainability. In the present study, at 30 and 37 days after sowing, plants fertilized with the lowest wood ash dose (4 g dm^{-3}) had, on average, lower than 6 mm stem diameters. However, it has not compromised ornamental sunflower inflorescence support. For leaf number, it was observed that during the experiment, plants subjected to wood ash treatments produced approximately 7 to 15 leaves (Figure 8).

At 30 days after sowing, the largest leaf number (14.67)

A

$y = -0.343333 + 0.841458***X - 0.027969***X^2$
$R^2 = 0.7412$

B

$y = 0.231336 + 0.352526***X - 0.011887***X^2$
$R^2 = 0.9466$

C

$y = 0.247338 + 0.249868***X - 0.008737***X$
$R^2 = 0.8469$

D

$y = 0.987151 + 1.117884***X - 0.0395** X^2$
$R^2 = 0.4646$
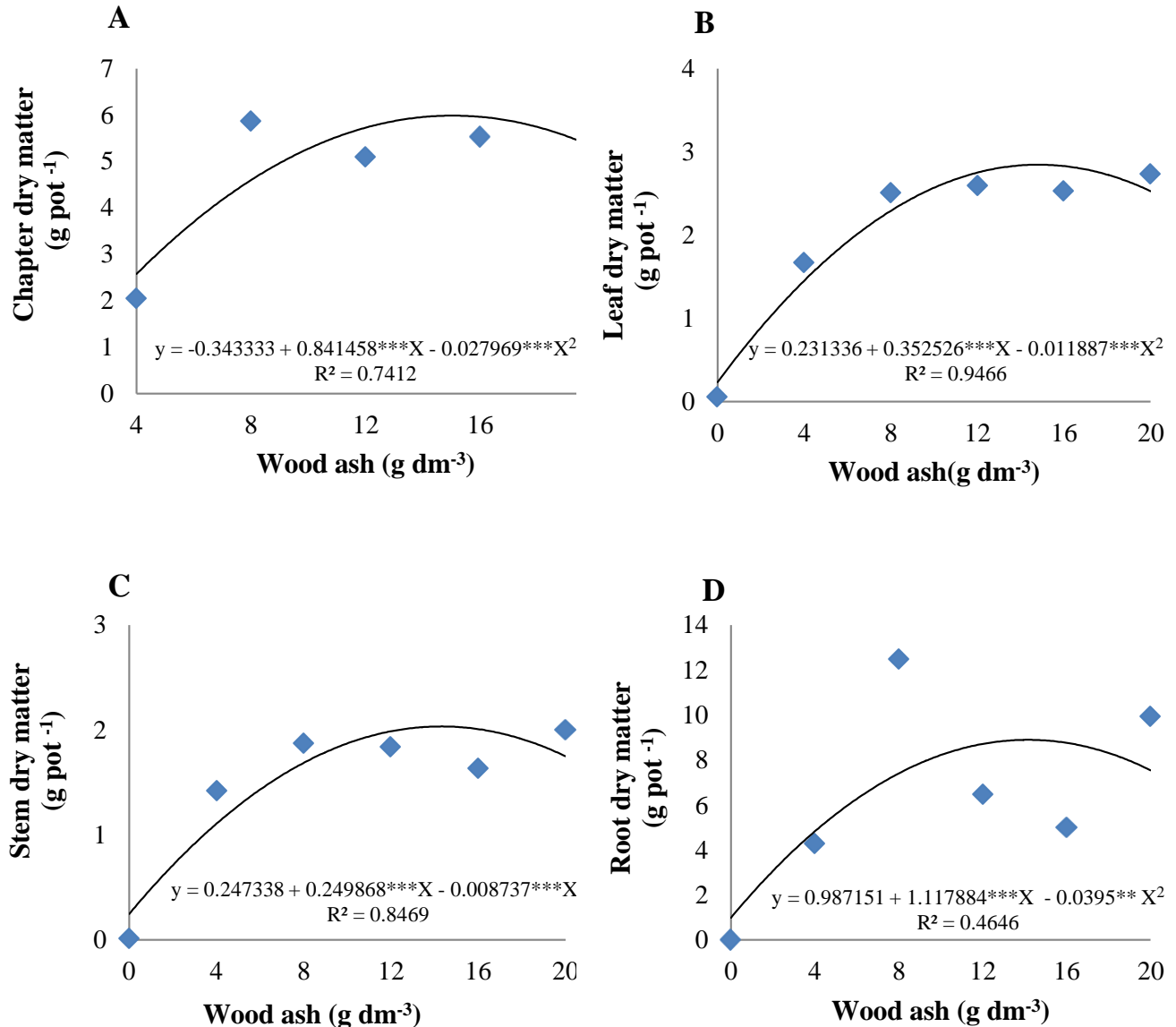
Figure 9. Ornamental sunflower chapter (A), Leaf (B), stem (C) and root (D) dry matter at 51 days after sowing influenced by wood ash doses. ***, ** 0.1 and 1% significance.

was observed with the 15.72 g dm^{-3} wood ash dose, obtaining 47.3% increase compared to the treatment without wood ash fertilization. At 37 days after sowing, Sunflower plant that received 14.25 g dm^{-3} of wood ash dose obtained the highest leaf number, giving an increase of 35.3%. From 44 days after sowing, it was observed that the number of leaves did no increase until the end of the experiment. With 14.76 g dm^{-3} being the wood ash dose that had the highest leaf number, with observed increase of 36.1%.

Leaf number is directly related to the plant leaf area, that is, the higher the leaf number, the higher will be the leaf area, thus being an important index in nutrition and plant growth studies, determining dry matter

accumulation, plant metabolism, potential photosynthetic capacity, yield and crop quality (Jorge and González, 1997; Oliveira et al., 2013).

In this study, leaves maximum number of 15 leaves in relation to wood ash doses is similar to the results observed by Sato et al. (2010) who found an average of 14 leaves per plant while assessing organic waste in substrate composition and ornamental sunflower development. For root, stem, leaf and the chapter dry matter was observed adjustment for the quadratic regression model. However, root dry matter showed a low determination coefficient (Figure 9D). As for chapter dry matter accumulation, there was a 57% increase in plants grown with a 15.04 g dm^{-3} wood ash dose when

A

$$y = 22.935 - 0.2079^{***} X$$
$$R^2 = 0.7838$$

B

$$y = 24.296667 - 0.284167^{***}X$$
$$R^2 = 0.8954$$
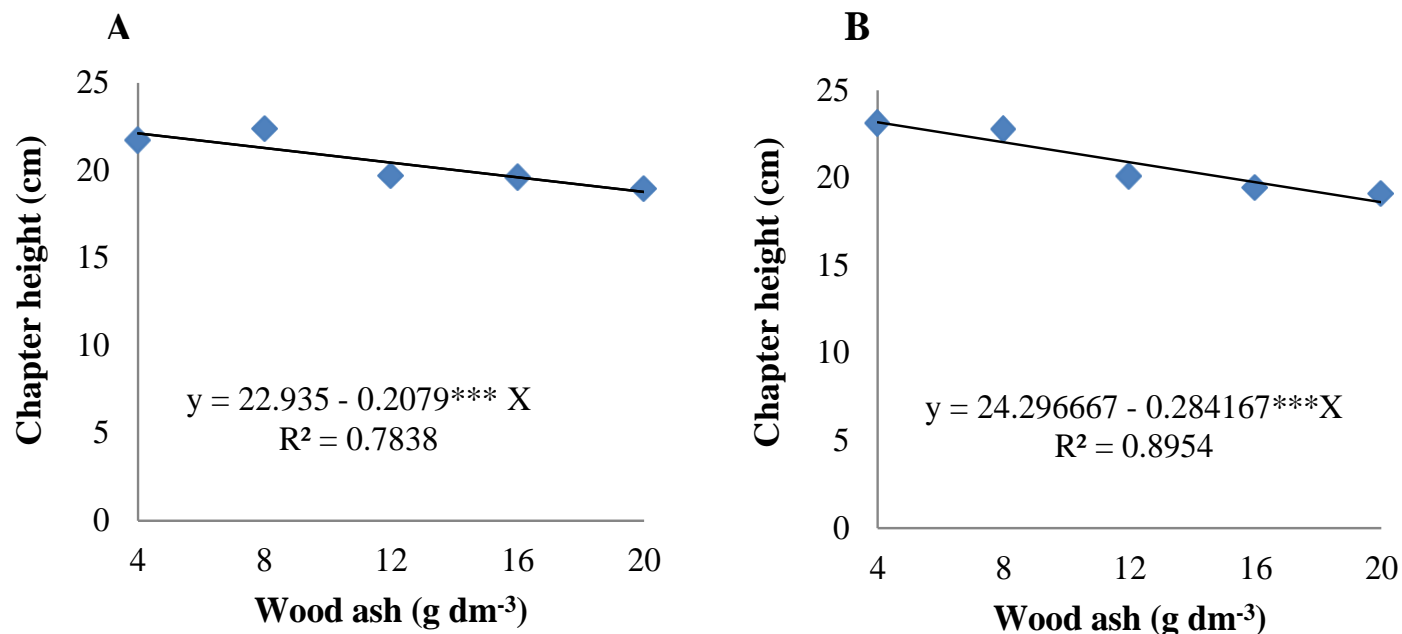
Figure 10. Ornamental sunflower chapter height at 44 (A) and 51 (B) days after sowing influenced by wood ash doses.***0.1% significant.

compared to dry matter production of plants fertilized with 4 g dm^{-3} wood ash (Figure 9A). For leaf dry matter, it was observed that the highest production of 2.85 g plant^{-1} was obtained with the 14.83 g dm^{-3} wood ash dose (Figure 9B), having an 91.9% dry matter production increase when compared to the treatment with no wood ash fertilization. Stem dry matter of plant that received 14.3 g dm^{-3} wood ash dose was higher than other doses, with an increase of 84.69% compared to the treatment that had not received wood ash fertilization (Figure 9C). Root dry matter (Figure 9D) highest production (8.90 g plant^{-1}) was obtained with the 14.15 g dm^{-3} wood ash dose, which had an increase of 88.9% when compared to the production of plant that did not receive wood ash fertilization.

Dry matter accumulation is a parameter that allows discussing plants organic translocation process (Benincasa, 2003). Thus, higher investment for root dry matter production was observed, followed by chapter, leaves and stems dry matter production, respectively (Figure 10). For ornamental sunflower chapter height at 44 and 51 days, results were fitted to a linear regression model. The highest values were observed in plant that received 4 g dm^{-3} wood ash dose, with 22.1 and 23.16 cm, respectively, for the two assessments.

Chapter height decreased inversely with wood ash doses. In the absence of wood ash application, sunflower did not produce a chapter (Figure 10). As noted in the plant height variable, commercially, the lower the plant height, better their commercial value is, providing a more pleasing aesthetics and portability. Therefore, values found in this study are meeting commercial standards for

ornamental sunflower plants.

According to Dallago (2000), wood ash fertilization significantly influence sunflower growth days after plants sowing, causing an increase reduction both in the lack or excess of nutrients. This result proves nutrients balance and soil pH importance in plant metabolism. Chapter diameter results at 44 and 51 days after sowing were set to the quadratic regression model. Wood ash doses that showed higher chapter diameter (10.48 and 10.95 cm) for the two assessments, respectively, were 13.2 and 13.7 g dm^{-3}. Increases at 44 and 51 days after sowing were of 73.9 and 23.6%, respectively (Figure 11).

Chapter diameter, along with plant height, has great influence on ornamental sunflower commercial value (Neves et al., 2005). For Sakata Seed Corporation (2003), inflorescence diameter values must be, on average, between 10.0 and 15.0 cm. These values can be obtained with wood ash use as corrective and fertilizer. According to Anefalos and Guilhoto (2003) and Dasoju et al. (1998), for potted ornamentation, sunflower plant chapter length is of utmost importance, as it should be proportional to the pot in which it is produced and marketed size. That is why stem final height reduction cannot drastically reduce chapter length, as it would lose commercial value. For sunflower disc diameter at 44 and 51 days after sowing, there was quadratic regression model adjustment (Figure 12). In these assessments, 5.25 and 6.51 cm values for disc diameter were obtained with 14.31 and 14.79 g dm^{-3} doses, respectively. At 44 days after sowing, there was a 60.4% disc diameter increase compared to the lowest assessed dose (4 g dm^{-3}),

A

B
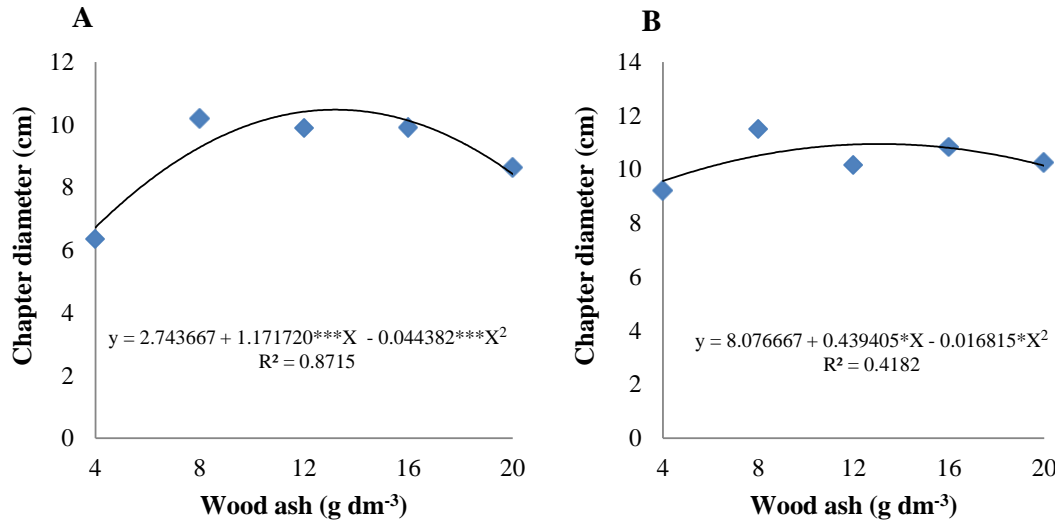
Figure 11. Ornamental sunflower chapter diameter at 44 (A) and 51 (B) days after sowing in relation to wood ash doses.***, * 0.1 and 5% significance.

A

B
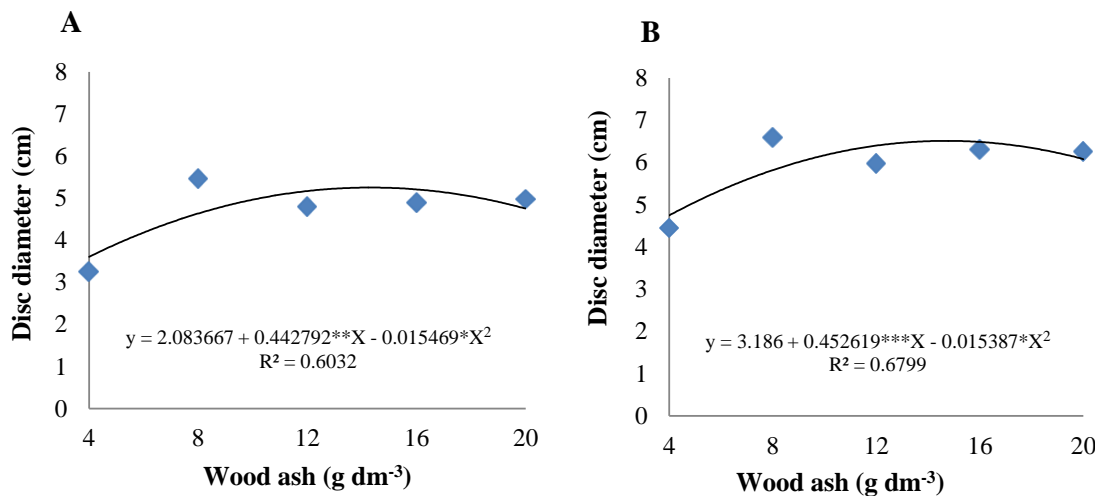
Figure 12. Wood ash effect on disc diameter at 44 (A) and 51 (B) days after sowing in. ** 1% significance level.

and at 51 days after sowing there was an increase of 51% (Figure 12). Comparing the tendency of disc diameter and chapter diameter curves at 44 and 51 days after sowing the respective periods, it was observed that there was similarity between them. Wood ash doses that provided higher chapter and disc diameters have close values. Thus, plants with larger chapter diameter, consequently, have larger disc diameter.

Conclusions

Doses of wood ashes between 12 and 16 g dm^{-3} may be

used to improve the development of ornamental sunflower plants, proving their effects as corrective and fertilizer for growing flowers.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Investigation of morphological indices of different rice varieties (*oryza sativa*) in relation to insect-pest attack under shallow and semi-deep land condition during *Kharif* Season

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Totally 23 different rice cultivars were analyzed for pest attack and varying morphological studies from Chinsurah, West Bengal, India. The individual rice cultivars were planted in both shallow and semi-deep conditions, maintaining the two replicated yield trail (RYT) with randomized block design (RBD) pattern. It was observed that the dead heart (DH) disease caused by *Scirpophaga incertulas* affects mostly NDR 8027 rice cultivar, which had maximum tillers in both shallow and semi deep conditions. The OR 2329-2 was less affected by DH, having minimum tillers in both shallow and semi deep conditions. The dead heart damages were directly proportional to the tillering stage and reproductive stage of rice plant. The mean value of pest attack in semi-deep land situation (SDW) (6.62%) was lesser than the mean value of pest attack in a shallow land situation (SHW) (6.68%). When compared the plant height of all 23 rice cultivars in shallow land was shorter than semi-deep land. From statistical analysis, it was understood that the rice cultivars of semi-deep land areas are less affected by insect-pest damage than the rice varieties of the shallow land area during the *Kharif* season. The rice cultivars grown in semi-deep land with high water depth were less damaged by pest and the plant heights of semi-deep land area is higher than the plants of the shallow land area. From this study, we concluded that the rice cultivation in semi-deep land condition is more beneficial than shallow land condition.

Key words: Rice cultivar, *Scirpophaga incertulas*, plant morphology, *Kharif* season, insect-pest damage.

INTRODUCTION

Rice (*Oryza sativa*) is a plant of Asian origin and native formers account for 87% of the world's total rice production (MacLean et al., 2002). Rice is the major food for half of the world's population and it supplies about 27% of dietary energy and 20% of dietary protein in the developing countries (WRS, 2010). In India, totally 44.3 million hectares of land are used for rice cultivation and stands second in rice production of around 89.09 million tons/ year (MacLean et al., 2002). The genus *Oryza* includes twenty wild species and two cultivated species

such as *O. sativa* (Asian rice) and *Oryza glaberrima* (African rice). Among this rice variety, *Oryza sativa* is the most commonly grown species throughout the world today (Parvez et al., 2006). Food shortages in developing countries are aggravated by rapid population growth. This rising population has made it more necessary to go in for increased agricultural production. The increase in rice production depends on the condition of the land where it is cultivated (Anusha and Sourik, 2014). On the other hand the insect pest attacks are the major biotic constraints in rice production. It is estimated that 25% of rice yield loss in India was reported due to the pest attack (Neeraj et al., 2013). Specifically, the rice variety *O. sativa* is attacked by Yellow stem borer (dead heart) by *Scirpophaga incertulas* (Anusha and Sourik, 2014). The level of many pests attacks significantly rises due to increased nitrogen levels and water may also influence the growth of some pest species (Rao et al., 1987). It has become a notable challenge to overcome the issues of pest attack to meet the increasing demand for rice. Hence, effective insect pest management strategies for rice plants are needed to be developed and implemented. So, the present study is to analyze the morphological indices of different rice plant varieties and their relationship with the insect-pest attack in different cultivation systems during *kharif* season.

MATERIALS AND METHODS

The field experiments are conducted during *Kharif* season in rice research station, Chinsurah, Hooghly, West Bengal. Latitude: 22°52' N. Longitude: 88°24' E. Elevation: 8.62 m above mean sea level (AMSL).

Rice cultivation systems

Totally 23 individual rice cultivars were planted in both shallow land condition (with water stagnation from 20 to 40 cm water depth, with capacity to tolerate sudden submergence for 7 to 10 days) and semi-deep condition (water stagnation from 40 to 75 cm water depth, with capacity to tolerate sudden submergence for 7 to 10 days) maintaining the two replicated yield trial (RYT) with randomized block design (RBD) pattern. All the 23 varieties of rice cultivars were planted in three rows with 20 cm spacing between each plant. All the rice varieties growth was observed and noted in both land conditions. All the varieties were observed their pest attack with a hand lens after trans-planting date (DAT) of 60th, 75th, 90th day respectively. A standard formula was used to calculate the dead heart damage percentages caused by *S. incertulas*.

$$\text{Dead Heart (DH\%)} = \frac{\text{No. of DH in 10 hills}}{\text{Total No. of tillers in 10 hills}} \times 100$$

Comparisons were made between damages observed in rice cultivars due to pest attack along with morphological changes, viz., a) Total No. of tillers, b) Flag leaf length (cm), c) Flag leaf breath (cm), d) Plant height (cm), e) Total No. of Panicle bearing tillers f) Leaf length (cm), g) Leaf breath (cm), h) Panicle length (cm). The data was collected for calculating levels of pest damage caused

by various pests during growth of the rice cultivar at 60th, 75th, 90th, 105th day's respectively from the date of transplanting (DAT).

RESULT AND DISCUSSION

In the present study, two different land conditions were used to investigate the level of growth and pest damage on 23 different rice cultivars. The rice varieties grown in semi-deep land conditions (SDW) showed better growth under water stagnation from 40 to 75 cm water depth and had the capacity to tolerate submergence for 7 to 10 days than shallow land condition with 20 to 40 cm of water stagnation (Table 1). The varieties grown in this condition (SDW) had prominent submergence tolerance and elongation ability. Similarly, the rice cultivation in semi-deep land condition was found to be more fruitful than shallow land condition (Anusha and Sourik, 2014).

The field investigation against pest damage was carried out during the *kharif* season. Studies showed that the NDR 8027 rice variety was highly affected with dead heart disease caused by *S. incertulas* (Figure 1) in the mean average range of 7.47% in SHW and 7.41% in SDW and the OR 2329-2 showed a minimum of 5.93% in SHW and 5.87% in SDW (Table 2). Similarly, the rice plants from the seedling to maximum tillering stages were attacked at the base of the stem had dead hearts attack (Tripathi et al., 1997). In the present study, less number of tillers were observed in both shallow and semi deep conditions. The mean average percentage of pest attack in SDW is less than the mean average percentage of pest attack in SHW was recorded. The earlier study reported that critical infestation of the stem borer occurred during vegetative and panicle stages of the *kharif* season (Parwez et al., 2006). It was also observed that the plant height of individual 23 rice cultivars in SHW land condition was shorter than SDW land condition. On a comparison analysis between dead heart damage of 23 rice cultivars in both shallow and semi-deep conditions the mean damaging average percentage of shallow land was 6.68% and semi-deep land was 6.62% (Table 2). So, the damaging effect of DH% in shallow land was higher (0.06%) than semi-deep land. Studies also revealed that higher water depth was reason for the lesser pest attack in semi-deep land conditions.

All the 23 rice cultivars grown in shallow condition, the parameters such as plant height, Total no. of tillers, leaf length, leaf breath, flag leaf length, flag leaf breath, panicle length and total number of panicle bearing tillers ware analyzed. Among this, the highest plant height was observed as 138.0 cm in RAU 1407-7-1-3-4 rice variety and the lowest was observed as 95.4 cm in Swarma-Sub1. The NDR 8027 has the highest (133) number of tillers and OR 2329-2 has the lowest number (96) of tillers. The highest leaf length was observed in LPR 1131 (20.5 cm) and the lowest was observed in NDR 9460 (13.8 cm). The highest leaf breath was observed in RAU 1407-7-1-3-4 (1.38 cm) and the lowered in Swarna-Sub1

Table 1. Rice cultivars planted in semi-deep condition and shallow land condition with different level of water stagnation.

S/N	Rice varieties	Plant height (avg.) in cm	
		Shallow land condition (20 to 40 cm water depth)	Semi-deep condition (40 to 75 cm water depth)
1	OR 2329-2	104.0	109.0
2	OR 2165-5	117.0	122.0
3	NDR 8027	105.7	110.7
4	NDR 8692	106.0	111.0
5	NDR 9460	105.6	110.6
6	NDR 9467	128.7	133.7
7	NDR 8850	128.1	133.1
8	NDR 9440	132.0	137.0
9	RAU 1407-7-1-3-3	115.0	120.0
10	RAU 1407-7-1-3-4	138.0	143.0
11	IR 70153-11-TTB 1-5	120.0	125.0
12	IR 70153-11-TTB 1-6	109.4	114.4
13	LPR 1112	107.0	112.0
14	LPR 1130	132.4	137.4
15	LPR 1131	117.9	122.9
16	CR 2415-3-2-1-1-1-1	130.0	135.0
17	CR 2754-18-6	105.4	110.4
18	CR 2750-6-2	129.0	134.0
19	Swarna	97.0	102.0
20	Swarna-sub1	95.4	100.4
21	Savitri	107.6	112.6
22	Savitri-sub1	108.8	114.8
23	CN 1039-9	103.3	108.3

Figure 1. Dead heart damage caused by *S. incertulas.*

Table 2. Comparison of dead heart damage between shallow (SHW) and semi-deep land (SDW) during *kharif* season.

S/N	Rice varieties	Mean DH% of RYT-SHW	Mean Avg. (%)	Mean DH% of RYT-SHW	Mean Avg. (%)
1	OR 2329-2	5.93		5.87	
2	OR 2165-5	6.83		6.77	
3	NDR 8027	7.47		7.41	
4	NDR 8692	7.31		7.25	
5	NDR 9460	5.94		5.88	
6	NDR 9467	6.29		6.23	
7	NDR 8850	6.49		6.43	
8	NDR 9440	6.30		6.24	
9	RAU 1407-7-1-3-3	6.81		6.75	
10	RAU 1407-7-1-3-4	6.91		6.85	
11	IR 70153-11-TTB 1-5	6.04		5.98	
12	IR 70153-11-TTB 1-6	6.55	**6.68**	6.49	**6.62**
13	LPR 1112	6.95		6.89	
14	LPR 1130	6.85		6.79	
15	LPR 1131	6.83		6.77	
16	CR 2415-3-2-1-1-1-1	5.95		5.89	
17	CR 2754-18-6	7.21		7.15	
18	CR 2750-6-2	7.33		7.27	
19	Swarna	6.74		6.68	
20	Swarna-sub1	6.32		6.23	
21	Savitri	7.01		6.95	
22	Savitri-sub1	6.72		6.66	
23	CN 1039-9	7.04		6.98	

(1.0 cm). The highest flag leaf length (18.5 cm) was observed in LPR 1131 and lowest in NDR 9460 (11.8 cm). The highest flag leaf breath was observed in RAU 1407-7-1-3-4 (1.36 cm) and lowered in Swarna-Sub1 (0.98 cm). The RAU 1407–7–1–3–4 has the longest panicle length (17.73 cm) and shortest panicle length in NDR 8027 (12.93 cm). The highest number of panicle bearing tillers (34) was observed in RAU 1407–7–1–3–4 and lowered in NDR8027 (Table 3). The range of parameters was differed from season to season (Anusha and Sourik Ghosh, 2014).

In semi-deep condition, among all 23 cultivates the highest plant height was observed in RAU 1407-7-1-3-4 (143.0 cm) and lowered in Swarna-Sub1 (100.4 cm). The highest total No. of tillers was observed in NDR 8027 (131) and lowered in 2329-2 (94). The highest leaf length was observed in LPR 1131 (20.2 cm) and lowered in NDR 9460 (13.5 cm). The highest leaf breath was observed in 1.36 cm of RAU 1407-7-1-3-4 and the lowest is 0.97 cm of Swarna-Sub1. The highest flag leaf length was observed in LPR 1131 (18.2cm) and lowest in NDR 9460 (11.5 cm). The highest flag leaf breath was observed in RAU 1407-7-1-3-4 (1.34 cm) and lowered in Swarna-Sub1 (0.95 cm). The longest panicle length was observed in RAU 1407–7–1–3–4 (17.70 cm) and it was lowered in NDR 8027 (12.90 cm). The highest total

number of panicle bearing tillers was observed in RAU 1407–7–1–3–4 (31) and the lowest number was in NDR 8027 (4) (Table 4). Similarly, the previous study reported that the yellow stem borer *S. incertulas* produced two broods, with the first peaking during the last week of September and the second peaking during the second week of November which coincided with the dough stage of rice (Tripathi et al., 1997).

Conclusion

Apart from being a staple food, rice has a historical significance and cultural relevance in the state of West Bengal. The results of the present study focus on the research and development initiatives, specifically on insect-pest management and growth. From the field investigation, it showed that the dead heart (*S. incertulas*) damages are directly proportional to the tillering stage and reproductive stage of rice plant. The rice cultivars of semi-deep land is less affected by insect-pest damage than the rice varieties of shallow land during the *Kharif* season. It also stated that due to high water depth, the cultivars of semi-deep land were less damaged by pest and also the plant heights of shallow land were shorter than the plants of semi-deep land. So, it was concluded

Table 3. Measurements of plant height, total No. of tillers, leaf length, leaf breath, flag leaf length, flag leaf breath, Panicle length, total No. of panicle bearing tillers in shallow condition.

S/N	Entries of RYT in shallow land condition	Plant height (cm)	Total no. of Tillers	Leaf length (cm)	Leaf breath (cm)	Flag leaf length (cm)	Flag leaf breath (cm)	Panicle length (cm)	Total no. of Panicle bearing tillers
1	OR 2329-2	104.0	96	14.3	1.07	12.3	1.05	13.43	08
2	OR 2165-5	117.0	111	18.3	1.10	16.3	1.08	13.83	10
3	NDR 8027	105.7	133	14.4	1.30	12.4	1.28	12.93	06
4	NDR 8692	106.0	128	17.9	1.04	15.9	1.02	16.73	26
5	NDR 9460	105.6	97	13.8	1.01	11.8	0.99	15.53	19
6	NDR 9467	128.7	101	15.4	1.33	13.4	1.31	14.43	15
7	NDR 8850	128.1	104	19.8	1.18	17.8	1.16	15.63	21
8	NDR 9440	132.0	102	15.0	1.13	13.0	1.11	15.03	13
9	RAU 1407-7-1-3-3	115.0	109	16.2	1.24	14.2	1.22	17.33	31
10	RAU 1407-7-1-3-4	138.0	113	18.2	1.38	16.2	1.36	17.73	34
11	IR 70153-11-TTB 1-5	120.0	99	14.0	1.19	12.0	1.17	17.13	30
12	IR 70153-11-TTB 1-6	109.4	105	14.7	1.05	12.7	1.03	16.93	28
13	LPR 1112	107.0	116	18.0	1.09	16.0	1.07	13.63	09
14	LPR 1130	132.4	112	16.0	1.27	14.0	1.25	14.63	11
15	LPR 1131	117.9	110	20.5	1.12	18.5	1.10	14.83	12
16	CR 2415-3-2-1-1-1-1	130.0	98	18.4	1.11	16.4	1.09	16.33	25
17	CR 2754-18-6	105.4	125	15.6	1.06	13.6	1.04	15.83	22
18	CR 2750-6-2	129.0	130	19.0	1.23	17.0	1.21	16.23	24
19	Swarna	97.0	108	17.2	1.03	15.2	1.01	15.13	18
20	Swarna-sub1	95.4	103	14.8	1.00	12.8	0.98	14.73	16
21	Savitri	107.6	118	17.7	1.02	15.7	1.00	13.13	07
22	Savitri-sub1	108.8	107	18.7	1.17	16.7	1.15	14.93	17
23	CN 1039-9	103.3	120	18.5	1.08	16.5	1.06	15.33	13

Table 4. Measurements of plant height, total No. of tillers, leaf length, leaf breath, flag leaf length, flag leaf breath, total panicle bearing tillers in semi-deep condition.

S/N	Entries of RYT in semi-deep condition	Plant height (cm)	Total No. of tillers	Leaf length (cm)	Leaf breath (cm)	Flag leaf length (cm)	Flag leaf breath (cm)	Panicle length (cm)	Total No. of Panicle bearing tillers
1	OR 2329-2	109.0	94	14.0	1.05	12.0	1.03	13.4	6
2	OR 2165-5	122.0	109	18.0	1.09	16.0	1.07	13.8	8
3	NDR 8027	110.7	131	14.1	1.28	12.1	1.26	12.9	4
4	NDR 8692	111.0	126	17.6	1.02	15.6	1.00	16.7	24
5	NDR 9460	110.6	95	13.5	0.99	11.5	0.97	15.5	17
6	NDR 9467	133.7	99	15.3	1.31	13.3	1.29	14.4	13
7	NDR 8850	133.1	102	19.5	1.16	17.5	1.14	15.6	19
8	NDR 9440	137.0	100	14.7	1.25	12.7	1.23	15.0	11
9	RAU 1407-7-1-3-3	120.0	107	15.9	1.08	13.9	1.06	17.3	30
10	RAU 1407-7-1-3-4	143.0	111	17.9	1.36	15.9	1.34	17.7	31
11	IR 70153-11-TTB 1-5	125.0	97	13.7	1.17	11.7	1.15	17.1	28
12	IR 70153-11-TTB 1-6	114.4	103	14.4	1.03	12.4	1.01	16.9	26
13	LPR 1112	112.0	114	17.7	1.07	15.7	1.05	13.6	7
14	LPR 1130	137.4	110	15.7	1.23	13.7	1.21	14.6	9

Table 4. Contd.

15	LPR 1131	122.9	108	20.2	1.13	18.2	1.11	14.8	10
16	CR 2415-3-2-1-1-1-1	135.0	96	18.1	1.10	16.1	1.08	16.3	23
17	CR 2754-18-6	110.4	123	15.3	1.04	13.3	1.02	15.8	20
18	CR 2750-6-2	134.0	128	18.7	1.21	16.7	1.19	16.2	22
19	Swarna	102.0	106	16.9	1.01	14.9	0.99	15.1	16
20	Swarna-sub1	100.4	101	14.5	0.97	12.5	0.95	14.7	14
21	Savitri	112.6	116	17.4	1.00	15.4	0.98	13.1	5
22	Savitri-sub1	114.8	105	18.4	1.15	16.4	1.13	14.9	15
23	CN 1039-9	108.3	118	18.2	1.06	16.2	1.04	15.3	12

that the rice cultivation in semi-deep land condition is more favorable and may also economically profitable and beneficial than shallow land condition.

Conflict of Interest

We declare that we have no conflict of interest.

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Full Length Research Paper

Obtaining physical-chemical analysis of the alcoholic distillate of cajarana (Spondias sp) in semiarid Paraiba

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This study aimed to produce, and consequently create physical-chemical analysis of alcohol distilled from the fruits *of Spondias* sp. The study was carried out at the Federal University of Campina Grande - Campus de Patos-PB (UFCG). 36.85 kg of cajarana and 1100 ml (869 g) of distilled alcohol, with a yield of 2.36% were collected, the cajarana used for this study presents equal 11.02° brix. This occurs with pH (2.74), being the necessary correction of the pH, to 4.5. During the production process of alcoholic distillate, the fermentation was achieved in vats steel with a capacity of 30 L using commercial mark Fleischman containing yeast of *Saccharomyces cerevisiae* strain, followed by fermentation kinetics. The alcoholic distillate showed high concentration of copper and methanol, the volatile acidity and higher alcohols were above normal, while the other components are in compliance with the law. For the analyses, methodology (MAP) Ministry of Agriculture, Livestock and Supply was used.

Key words: Fruits of the caatinga, *saccharomyces cerevisiae* fermentation, distillate.

INTRODUCTION

The history of alcohol goes side by side with the history of chemistry, because it has reports of the same fermentation process with that of ancient Egypt, with the same inhaled flavoring vapors when fermented. The artisans have worked in fermentation and distillation processes, but they did everything empirically.

Sugar cane is the main raw material for the production of cachaça in Brazil, but the "cachaça" can be produced from any plant or fruit rich in sucrose (sugar), fructose, glucose, cellulose, etc. In Italy, Grappa is produce, a grape distillate; in Germany, Kirsch, a cherry distillate;

Scotland, Whisky, sacrificed barley distilled; in Portugal, Brandy, distilled from grape pomace (Fernandes; Oliveira, 2010). As the Cajarana (Spondias sp), a fruit rich in sugar (fructose), can also produce a distilled alcohol by fermentation process. The Cajarana (Spondias sp) belongs to Anacardiácea family, originated in Central America, adapts to various types of soil and its fruits have a variety of major use in the production of pulp (ice cream, candy, juices, among others); It can be consumed raw or in the production of animal feed.

According to Silva et al. (1984), the Northeast region

presents a wide diversity of tropical fruits with good prospects for economic exploitation, although mostly present extractive character and commercialization restricted to fruit "in natura" or as juice or ice cream. The Spondias genus belongs to Anacardiaceae family, and has 18 species, some of which that occur in the Northeast (Spondias sp, *Spondias mombin* L, *Spondias purpurea, Spondias tuberosa*, L.) are tropical fruit trees in adaptation and of high commercial value (Mitchell, 1995). The mature fruit of Spondias has a high starch content and in some cases, one can even see the starchy taste in the fresh red mombin fruit. The total content of pectin is also high in comparison to most of the fruit, which together to the high starch content may impair the stability of juice (Melo, 2010).

The fruit fermentation process is followed by distillation were spirits is obtain, being necessary to adapt the production method according to the raw material. Decree No. 2314, of the MAP defines fruit spirit or brandy fruits as alcohol drink with 36 to 54°GL at 20°C, obtained from simple alcoholic distilled from fruit juice, or by the distillation of fermented mash fruit (Brazil, 1997). Yeasts of different strains of *Saccharomyces cerevisiae* were found in greater abundance in fermentation for the production of brandy (Morais et al., 1997) being the most distinctive by high alcohol yield and tolerance to high concentrations of ethanol.

The fermentation under controlled conditions involve chemical conversions. Some of the most important steps are: oxidation of ethanol to acetaldehyde (partial oxidation), and then acetic acid (total oxidation); from sucrose to citric acid and from dextrose to gluconic acid; reduction of aldehyde to alcohol (acetaldehyde to ethanol) and sulfur to hydrogen sulfide; hydrolysis of starch to glucose, and sucrose to glucose, fructose and alcohol; and esterification, hexose phosphate from hexose and phosphoric acid (Shreve and Brink Jr., 1977). The fermentation time ranges from 24 to 30 h, when the wine reaches 0° Brix (Lima, 1999; Faria, 1995). After that time, the yeast will settle to the bottom of the fermentation vat, and then the wine will be forwarded for distillation and a new wort vat would be added (Canton, 2006).

The pH is an important factor in the alcoholic fermentation process; it is recommended an initial pH values would be between 3.8 and 4.0; this pH range may be sufficient to allow rapid alcoholic fermentation and inhibit undesirable bacteria (Aquarone et al., 1983). The distillation of wine results in two fractions called phlegm (brandy) and vinasse. The first, which is the main product of distillation, consists of an impure water-alcohol mixture and vinasse, whose alcohol content should be zero, but it accumulate all fixed substances and part of volatile compounds (Nogueira and Venturini, 2005).

The type of distillery influences the content of volatile compounds. According to studies by Faria and Pourchet (1989) and Nascimento et al. (1998), cachaça distilled in copper destillery showed a higher aldehydes and methanol levels that the spirits distilled in stainless steel destillery, which in turn contained higher concentrations of higher alcohols and esters. After distillation, the cachaça must present an alcohol content between 38 to 48 GL (Miranda et al, 2007), and a quantity of a product "secondary" responsible for the characteristic flavor and odor (Almeida and Barreto, 1971; Lima, 1964; Yokoya, 1995).

The physical and chemical quality of the sugar cane brandy in Brazil is regulated by the Ministry of Agriculture (Brazil, 1997), which sets limits for various parameters (alcohol content, volatile acidity, esters, higher alcohols, aldehydes, furfural, methanol and copper). The fruit brandy follows the same law of cachaça, except for alcohol content limit which is 36 to 54°GL (Brazil, 2008).

The obtaining of distillate has a reasonable degree of complexity, and wide acceptance in domestic and international markets (because it is similar to cachaça, the distilled more consumed in Brazil), so this work aims to add value to the culture of Spondias, the man of the field in the Paraiba semi-arid region, study use of Cajarana as a potential for organic alcohol distillate production, with industrial purpose of analyzing their physical and chemical properties as well as its chemical composition, and describing their compliance with the standard identity and quality provided by law in Brazil in the production of beverages.

MATERIALS AND METHODS

For the experimental production of distilled alcohol, Cajarana (Spondia sp) was used with soluble solids content of 11.04°brix, produced in the Wood Chemical Laboratory of UFCG - Federal University of Campina Grande - Campus of Patos-PB. After each sample was taken, scavenging of the samples to remove unwanted material they were washed in solution 2% v / v for 30 min, rinsed with clean water and placed in a freezer at -15°C to maintain their chemical and bromatologic properties. The fruits were pulped and then centrifuged to remove excess of pectin, for this surplus implies a high concentration of methanol in distilled alcohol (Babylon, 2010).

Distillate of Cajarana (*Spondias* sp)

The alcohol distillate of Cajarana was fermented in stainless steel vats with a capacity of 30 liters of wort and copper stills with a capacity of 10 liters per cachaça making. The production of alcoholic distillate from Cajarana passes through five basic steps:

(1). Pulping, in which step removes the pulp of the fruit to initiate the whole process.
(2). Centrifuging the pulp to remove excess of pectin, since the pectin is responsible for the formation of methanol.
(3). Preparation of the wort, which is prepared from a mixture of Cajarana juice, which will be fermented and it will originate the cachaça, the fermentation and addition of yeast (Saccharomyces cerevisiae) to transform sugars into alcohol, carbon dioxide and other components.
(4). Distilling the beverage purification step, separating the

Figure 1. Preparation of fermentable wort in steel vat, up to 30 liters of wort.

unwanted components.
(5). Analysis of the distilled alcohol.

Fermentation

The preparation tank was made up of 150 g of Fleischmann's Yeast (lyophilized yeast) containing the yeast strain *S. cerevisiae* and sorbitan monostearate (a substance which facilitates dissolution and viscosity increasing ingredients) with 200 ml of water heated to 45°C and 200 ml of the "juice" of Cajarana, then adding 12.10 kg of the "juice." Fermentation took place in stainless steel fermentation vat, with capacity for thirty liters of fermentable wort (Figure 1).

Kinetics of fermentation

The fermentation started after the preparation of the tank's foot at ambient temperature, yeast, freeze-dried, of the Flechman brand, and the Cajarana wort were used, and they were prepared for fermenting for 24 h. During fermentation, monitoring was done and fermentation kinetics was used to analyzed alcohol content, ° Brix, pH and total acidity in all time intervals range from every hour, and after in two hours and finally at three hours.

The fermented alcoholic content was observed in ebulliometer 3300, with decimal determining the alcoholic strength, accompanied by a boiler with tap, protective tube, condenser and base in chromed steel, an alcohol lamp in aluminum, appropriate thermometer and cork latex, ruler in polypropylene with an alcohol content of scale 0 to 25°GL, sliding scale of 86 to 101°C, lock pin, cursor and graduated glass bucket. The Brix was found in automatic digital refractometer Acetec GDR 8600, at 20°C. The pH was checked at pH meter counters, digital ANALION PM 608. The total acidity was measured by volumetric titration method, a solution of 0.1 N sodium hydroxide was used, and as indicator,

phenolphthalein alcohol solution of 3% was also used (Brazil, 1986).

Distillation

Distillation was carried out in copper still, adding approximately 12 L of wine (fermented), 2.5° Brix and alcohol content of 4.25°GL.

Physico-chemical analysis of distilled alcohol

The analysis of Cajarana distillate were made in the ITEP (PERNAMBUCO TECHNOLOGY INSTITUTE - Waste Analysis Laboratory of Pesticides and Alcoholic Beverages - LABTOX) using the POP TC 020 and IT 006 method (Quality System Document Lab Tox), with the Bibliographical references: MAP / DAS / CGAL - Beverage and Vinegars Analysis Manual, accreditation of INMETRO (CRL 0153), signatory of mutual recognition agreement for certification accreditation body activity of products and management systems quality and environmental. D.O.U. - Section 1 - Edition # 124 of 30/06/2005: MAPA Normative Instruction 13 of 29/06/2005. D.O.U. - Section 1 - Issue Number 184, 25/09/2009: INMETRO Ordinance 276 of 24/09/2009.

The alcohol content was analyzed by Anton Paar densimeter (Figure 2A) 4500 DMA (v / v), and density calculated in g / cm3, acidity was analyzed in the electronic tag titrator METTLER TOLEDO / DL22 (Figure 2B) and copper and arsenic by Atomic Absorption Spectrometry in EAA-001 spectrometer; (Figure 2C) GFS97 AASpectrometer model.

Chromatography

Analyzes were made of furfural, aldehydes, esters, higher alcohols (n-propyl alcohol, isobutyl alcohol and alcohols isoamílicos) by

(A)   (B)   (C)
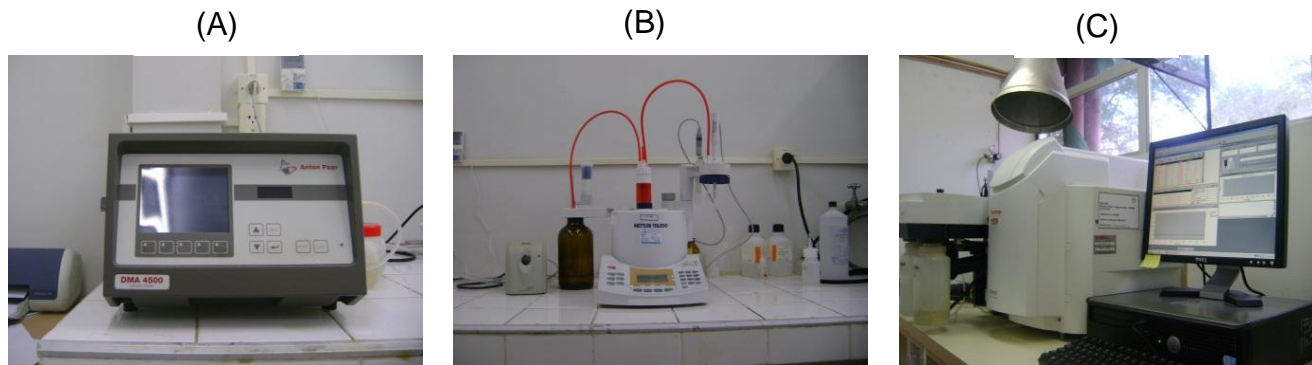
Figure 2. Equipment used for the analyzes of the alcoholic strength densimeter ANTON PAAR (a), acidity, titrator METTLER TOLEDO (b) and content of copper and arsenic Spectrometer AAS-001 (c).

(A)   (B)
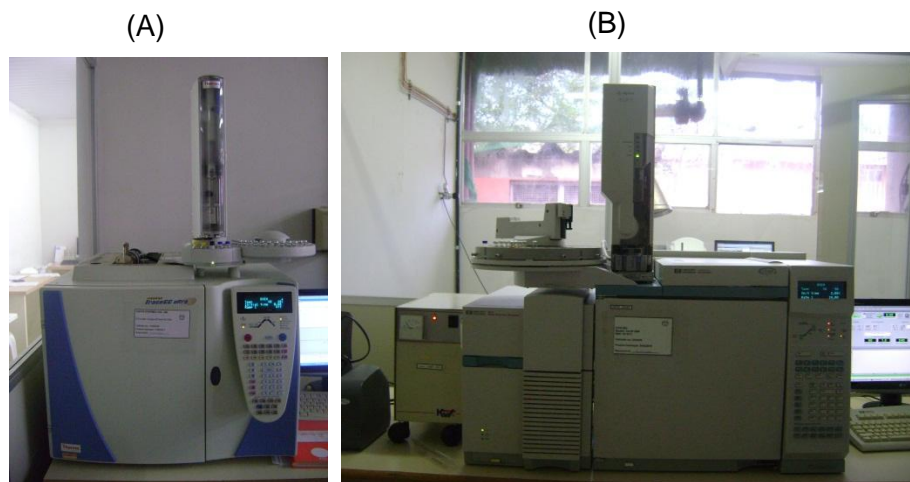
Figure 3. Chromatograph used for analysis of aldehydes, esters, higher alcohols (a) and ethyl carbamate (b).

means of gas chromatography (GC) using a gas chromatograph Varian CGC-006 with ionization detector FID flame, CARBOWAX 20M-type column (60 m x 0.25 mm x 1.0 microns), injector temperature of 230 and 250°C temperature detector, the volume of the injected sample of 1 μL, using as carrier gas Helium Ultra Pure with a flow of 1.5 ml / min patterns used in the ratio "SPLIT" 1:30 (Figure 3A). Also, chromatography was performed as the analysis of the concentration of ethyl carbamate using a Varian chromatograph GCC-003, model 6890 CGHP, MSD: HP 5973, column type CARBOWAX (60 m x 0.32 mm x 1 μm), injector temperature 24°C (SPLIR less) and sample volumes of 1 μL injection (Figure 3B).

RESULTS AND DISCUSSION

Physical and physico-chemical analysis of the fruit

Table 1 shows the results of physico-chemical analyzes. The analyzed fruits had an average length of 20.11 mm and weighing 9.78 g, and the length / weight 2.06 mm / g.

Kinetics of fermentation

During the first 12 h the fermentation was maintained in progress with ºbrix always decreasing, until the Brix remained constant during the remaining 12 h of fermentation. The initial Brix juice of the Cajarana, which is 11.04, is ideal for the fermentation process described by the literature, of Guangzhou (2006) ideally a ºBrix near 15. The ideal fermentation occurs in a range of 14 to 16°Brix, above this, fermentation becomes slow and incomplete (PATARO et al., 2002), whereas, less than 10°Brix (low) decreases the yield of distillate and facilitates infection of wort (Lima, 2001). The data obtained during the fermentation process are described in Table 2.

Table 1. Physico-chemical analysis of the pulp of Cajarana.

Analysis	Results
Carbohydrates (%)	9.59
Total protein (%)	0.75
Total lipids (%)	0.60
Acidity in citric acid (%)	1.39
Reducing sugars in glucose (%)	7.22
Nonreducing sugar in sucrose (%)	6.72
Total sugars (%)	13.94
Soluble solids in Brix degree (° Bx)	11.04
pH	2.74

Table 2. Analysis of Brix, alcohol content, pH and acidity during the fermentation process.

Hour	°Brix	Alcoholic content	pH	Total acidity (g/100 ml)
10h40	11.04	0	4.50	0.19
11h40	10.5	0.3	4.34	0.20
12h40	9.5	0.7	4.20	0.21
13h40	9	1.0	4.11	0.22
14h40	9	1.0	4.08	0.23
15h40	8	1.55	4.08	0.23
16h40	6.5	2.25	4.08	0.22
18h40	4	3.5	4.08	0.20
20h40	3	4.0	4.16	0.19
22h40	2.5	4.25	4.54	0.19
00h40	2.5	4.25	4.20	0.20
02h40	2.5	4.25	3.92	0.23
04h40	2.5	4.25	3.98	0.23
07h40	2.5	4.25	3.88	0.24
10h40	2.5	4.25	4.00	0.22
Mean	-	-	4.14	0.21

At the end of the fermentation process of this experiment, it is found that the sugar content (Brix) was not reset, which occurs within 24 to 36 h (Lima, 1999), this fact indicated that fermentation is not complete, one of the causes of this problem may be, the lower initial ºBrix of the wort (11.04). Graphic 1, express the results obtained in the fermentation, it appears that there was a decay in sucrose content (by lowering the Brix) during the first 12 h, and gradually increasing the alcohol content within 12 h if the final alcohol content remains constant because there is no more substrate consumption.

The lower pH was greater than 3.88 and 4.54, causing a pH change from 0.66 (ΔpH = 0.66) while the lowest total acidity was 0.19 g /100 ml and the greater was 0.24 g / 100 ml, there was a variation in the total acidity of 0.05 g/100 ml. The constant acidity indicates a good fermentation (Aquarone et al., 1975). The initial pH of the fermentation is 4.5. Studies of Aquarone et al. (1983) shows that the initial pH should be between 3.8 to 4.0, which allows a rapid fermentation, to Eiroa (1989) pH values below 4.5 development of bacteria.

Physico-chemical analysis of the fermentation

Comparing the fermentation data (pH and acidity) obtained in this study, the pseudo fermented cashew (Torres et al., 2006) and grapes for the production of red wine (Embrapa, 2000) (Table 3). The values of pH and acidity for the fermented Cajarana, corresponds to the arithmetic mean of all values observed throughout the fermentation process.

The fermentation of Cajarana occurs with low acidity and higher pH. The total acidity of wine should be in the range from 3.3 to 7.8 g / L (Rizzon et al., 1994). Observing Table 3, it is apparent that the fermented Cajarana presents a concentration of 2.1 g / L and less than the fermented concentration of cashew (7.2 g / L)
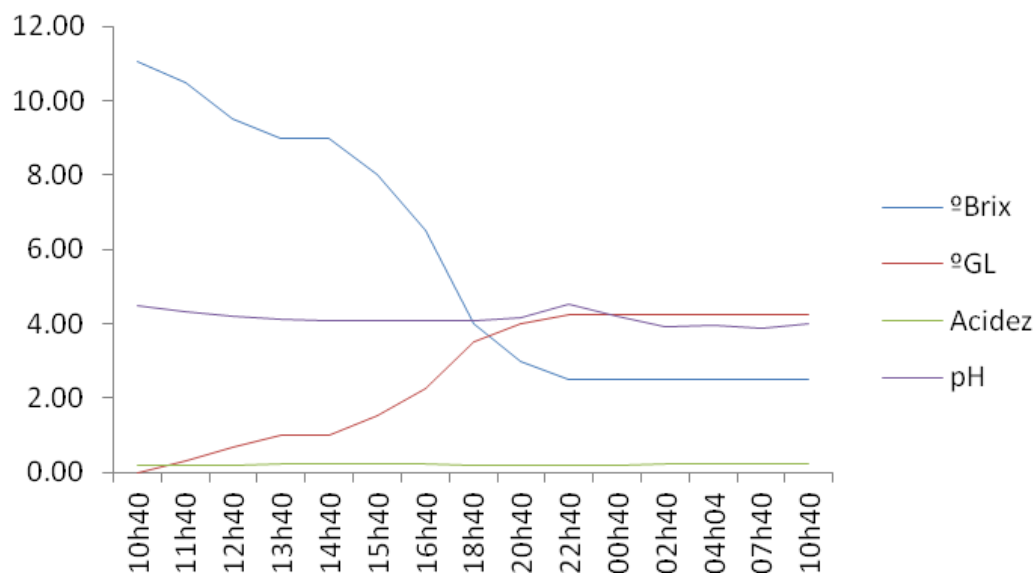
Graphic 1. Results of °Brix; °GL, pH and acidity as a function of time (h) obtained by the fermentation kinetics.

Table 3. PH and acidity in fermentation processes, of cajarana, cashew and grape.

Fermented	pH	Acidity (g/L)
Cajarana	4.14	2.1
Cashew	3.5	7.2
Grape	3.6	4.4

and grape (4.4 g / L). The initial pH of 4.14 of the fermented of Cajarana confers an increased resistance to infections or contaminations (Aquarone et al., 1983). The fermented of Cajarana has a pH equal to 4.14, this is due to lower total acidity in the fermented cashew (3.5) and grapes (3.6) (Torres et al., 2006; Brapa, 2000).

Statistical analysis

The evaluation of the fermentation kinetics was calculated using the correlation test of Pearson to the level of significance of 5% for the °brix and the alcohol content (Graphic 2). It is observed in Table 4 a negative correlation (Pearson r = -0.8888), its being observed in Figure 2 that fermentation becomes constant from the time of ¬720 min, with a membership around 79% (r = 0.7900). P value <0.0001 shows that there is a significant variation as it relates x fermentation time. For the alcohol content, a positive correlation (Pearson r = 0.8891) was observed. The 720 min did not observe further change, although there is a correlation around 79% (r = 0.7905).

P value <0.0001 shows that there is a significant variation in time when relating x-alcohol.

Distillation

880 ml (cm^3) of the heart fraction of distillate were obtained, with a density of 0.96529 g / cm^3 (Brazil 2005), that means that 849.45 g of liquor, with the amount of collected fruit 36.85 kg (36,850 g) in the final process had a yield of 2.31%, in correlation with the wine (12,060 g or 12493.65 ml), the yield was 7.04%, Cardoso (2001) found brandy sugar cane varies from 15 to 17% of the "wine" for brandy with 38 to 54% ethanol.

Physico-chemical analysis of the distillate

The data of the physico-chemical analysis for alcohol distillate of Cajarana are shown in Table 5, along with data to be compared with the spirit of jabuticaba in a study conducted by Asquieri et al. (2009), and the
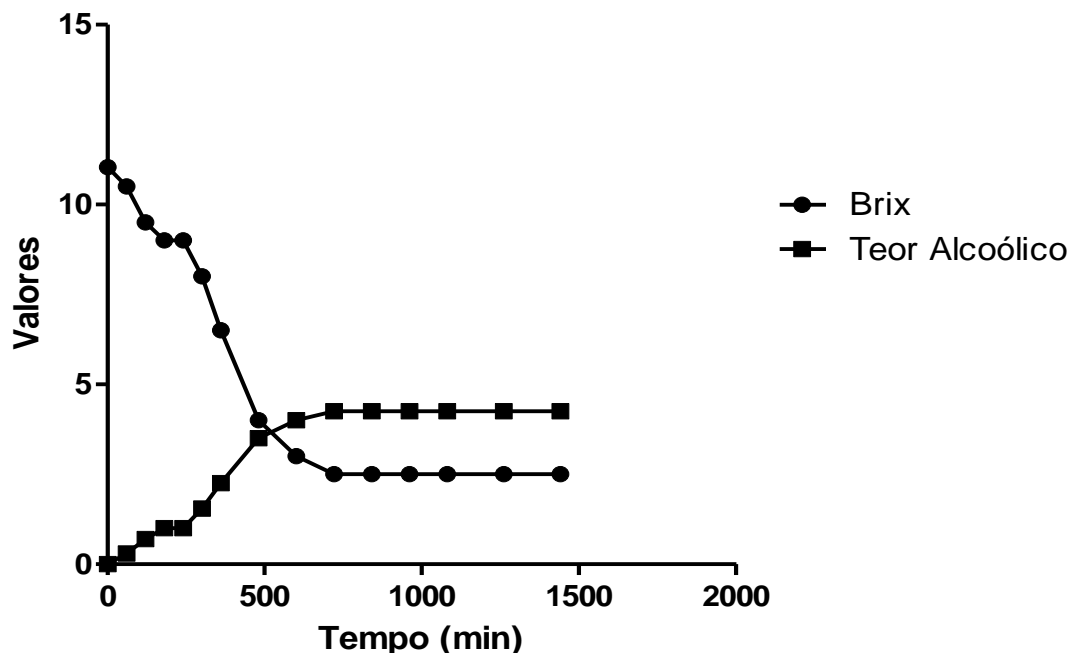
Graphic 2. Pearson correlation to the ºbrix and alcohol content.

Table 4. Data generated by the software Graphpad Prism, for the PEARSON correlation test.

Variables	ºbrix	Alcohol content
Number of XY Pairs	15	15
Pearson r	-0,8888	0,8891
95% confidence interval	-0,9628 to -0,6913	0,6920 to 0,9628
P value (two-tailed)	< 0,0001	< 0,0001
P value summary	****	****
Is the correlation significant? (alpha=0.05)	Yes	Yes
R square	0,7900	0,7905

analysis of Brazilian spirits and cachaça sugar cane made by Miranda et al. (2007) in parameter of quality of the Ministry of Agriculture, Livestock and Supply, according to Ordinance No. 65 of 23/04/2008, for fruit spirits.

Alcohol content

The alcohol content of distillate of Cajarana (28.94°GL) is below the minimum established by the Brazilian legislation for fruit brandy of 36 to 54°GL, (Brazil, 2008). The low alcohol content (28.94°GL) of this beverage is due to two factors:

(1). First, low °Brix of the fruit (11.04), which indicates that the result has a low amount of substrate (sugars) to be converted into alcohol. During the fermentation process, the sugars by the action of yeast are transformed into ethyl alcohol (Shreve and Brink, 1977), indicating that the amount of alcohol formed is directly proportional to the amount of sugars.

(2). Second, the Brix was not reset, leaving 2.5, that is beside the Cajarana present a small amount of sugars to be converted into alcohol, this small amount is not completely converted to alcohol. The fermentation ends between 24 and 30 h, when the wine reaches zero °brix (Lima, 1999; Do, 1995).

Concentration of furfural; ethyl carbamate; acrolein and arsenic

The concentration of each component was lower than that prescribed by law, as shown in Table 5, and the furfural values were higher than those found by works

Table 5. Comparative analytical data for distillate of Cajarana, jabuticaba and sugar cane.

Item to be analyzed	Tolerance	Mean values of distilled beverage of:		
		Cajarana	Jabuticaba	Cana-de-açúcar
Alcohol content	36º GL a 54º GL a 20 ºC	28.94	39	40.23
Copper	Máx. 5mg/L	52	0.70	2.57
Arsenic	Máx. 0,1 mg/L	<0.008	-	-
Sugar (sucrose)	Máx. 30 g/L	<1.0	0	-
Volatile acidity	Máx. 100 mg/100 ml	214	30	55.82
Total esters	Máx. 250 mg/100 ml	64.3	357	46.23
Aldehydes	Máx. 30 mg/100 ml	25	13.60	19.78
Furfural + hidroximetilfurf.	Máx. 5 mg/100 ml	<1.52	ND	0.11
Higher alcohols	Máx. 360 mg/100 ml	466.3	259.07	278.49
Congeners coefficient	200 a 650 mg/100 ml	769.4	659.67	400.46
Methanol	Máx. 20 mg/100 ml	62.1	4.3	8.53
Ethyl carbamate	Máx. 0,15 g/L	$<5 \times 10^{-5}$	-	-
Acrolein	Máx. 5 mg/100 ml	<1.31	-	-

done by Asquieri et al. (2009) and Miranda et al. (2007), which makes the beverage without carcinogenic compounds.

Methanol

The alcohol distillate of Cajarana has a methanol content of 62.1 mg / 100 ml, being above the maximum allowed by Brazilian law of 20 mg / 100 ml (Brazil, 2005), higher than the brandy of jabuticaba 4.3 mg / 100 ml (Asquieri et al., 2009) and higher than the analysis of Brazilian spirits in studies performed by Miranda et al. (2007), which is 8.53 mg / 100 ml. The high concentration of methanol in the distillate of Cajarana regarding the jabuticaba and sugar cane is due to the high concentration of pectin in Cajarana. Figure 4, shows that even after centrifugation, the limpid juicy is still cloudy which is also due to the high concentration of pectin since centrifugation is not sufficient for complete removal of pectin.

Total esters

The value found for the distillate of Cajarana, is 164.3 mg / 100 ml, this is below the maximum allowed by law, higher values were found for brandy of jabuticaba (Asquieri et el., 2009), which was 357 mg / 100 ml, and the analyzes for spirits and sugar cane cachaça, in a study conducted by Miranda et al. (2007), which was 46.23 mg / 100 ml. Reasonable concentration of esters in Cajarana distillate is responsible for its discrete characteristic aroma (Winhol, 1976), this may have occurred due to the low content of alcohol formed during fermentation, or according to Aquarone (2001) at a distillation rate slightly larger than the ideal.

Aldehydes

The value found for the distillate of Cajarana was 25 mg / 100 ml, lying close to the maximum allowed by Brazilian law, which is 30 mg / 100 ml (Brazil, 2005), which is a plus point. Values found by Asquieri et al. (2009) for jabuticaba spirits and Miranda et al. (2007), studies over Brazilian spirits, were respectively equal to 13.60 mg / 100 ml and 19.78 mg / 100 ml. The acetaldehyde is a compound that interferes negatively on brandy quality, because it can cause headaches, intoxication and problems related to the central nervous system (Cardoso, 1998).

Higher alcohols

The distillate of Cajarana has a concentration of higher alcohols of 466.3 mg / 100 ml, which is above the maximum limit established by Brazilian legislation of 360 mg / 100 ml and above jabuticaba Brandy values studied by Asquieri et al. (2009) which was 259.07 mg / 100 ml, and also above the values of spirits studied by Miranda et al. (2007) to 278.49 mg / 100 ml. This high strength must be connected directly to the fermentation process and the fermentation conditions (Giudici et al., 1990), since the concentration of ethyl alcohol was added to this low concentration of higher alcohols.

Volatile acidity

To volatile, acidity is allowed a maximum of 100 mg / 100 ml, for distillate of Cajarana was found a value of 214 mg / 100 ml, this value was still higher than those found by Asquieri et al. (2009), for brandy of jabuticaba 30 mg /
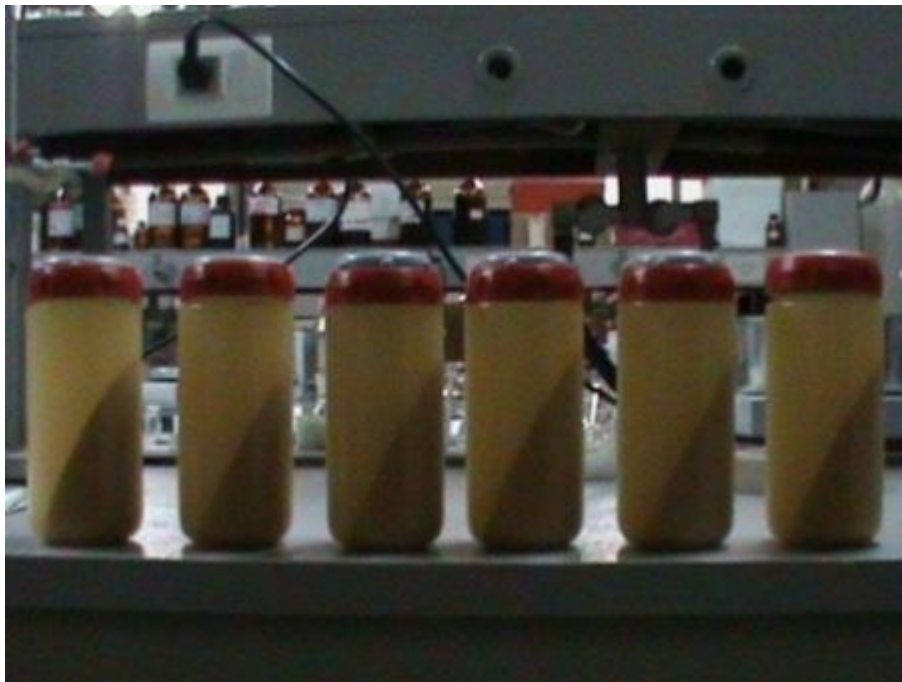
Figure 4. Cajarana centrifuged.

100 ml, and found by Miranda et al. (2009) for analysis of cachaça and spirits of sugar cane 55.82 mg / 100 ml. Acetic acid is the secondary component of greater responsibility for volatile acidity of Brandy (Lima, 1964; Nykamen and Nykamen, 1983).

Copper concentration

The concentration of copper in Cajarana spirit is ten times higher than the maximum set by the Brazilian legislation (Brazil, 2005), becoming irrelevant to compare this data with any other drink. This high copper concentration in the distillate may be due to the use of virgin copper which was first used for this study. Initial cleaning was carried out in three stages, first distiller was cleaned with soap and water washing, then washed again with lemon juice and finally was washed with pure water.

Conclusion

The production of Cajarana (Spondias sp), aiming to get alcoholic beverage, with a structured for the use of 100% of the fruit is certainly a viable process and very important for the semiarid of Paraiba. In pulping, the shell and core may be used for animal feed production, since they are rich in fiber. In centrifugation, pectin (the solid portion) can be used as a concentrated pulp for the production of juice, ice cream and others, as this keeps the mass and

good organoleptic properties of minerals and vitamins of the fruit.

The yeast-alcoholic beverage distilled from Cajarana presents moderate alcohol content (28.94°GL), which is less than the alcohol content of cachaça, brandy and whisky, and greater than that of wine and beer, and may even be considered a low spirits alcoholic. The high content of methanol in the alcohol distillate of Cajarana is due to high concentration of pectin. To decrease the amount of methanol, a bi-distillation of the drink can be realized, which has not been possible due to the small amount of distillate obtained in the first distillation, or an enzymatic process for the extraction of the pectin after centrifugation. The industries of alcoholic beverage production derived from fruit are growing and perfecting techniques to improve final product quality and increase productivity, the same should apply to the material in this study, which may receive additional studies to improve the final product.

Conflict of Interest

The authors have not declared any conflict of interest.

this study.

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Full Length Research Paper

Delineation and geographic information system (GIS) mapping of soil nutrient status of sugarcane growing tracts of Theni district, Tamil Nadu

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A global positioning system (GPS) based soil survey was conducted during September 2011 in the sugarcane dominant tracts of Theni district, Tamil Nadu to map the soil fertility status using GIS software and link with different cane yield potentials. The soil samples were taken from 400 spatially distributed locations from a depth of 0 to 30 cm and analyzed for texture, pH, EC, organic carbon, available nitrogen, phosphorus, potassium and micronutrients viz., Fe, Mn, Zn and Cu. The 'Asis' survey data regarding fertilizer management practices, adoption of new technologies, irrigation practices and other crop production techniques were also collected from the farmers. The interpolated maps for the status of macro and micronutrients show a clear deficiency of available Nitrogen in 96% of the soil samples of the cane growing areas and DTPA – extractable Zn deficiency in almost 60% of the regions surveyed. However, the available phosphorus and potassium contents were reported to be moderate to high in 90% of the samples indicating the possibility to reduce the P & K fertilizer application to sugarcane in this tract of Tamil Nadu. Majority of the soils were sandy loam to sandy clay loam in texture and reported to have low organic carbon context (<0.5%). The outcome of this study has provided an insight into the unbalanced fertilization practices adopted in some of the cane dominant tracts of this district and the need to evolve a GIS based balanced fertilization model through the conduct of field experiments with graded levels of fertilizer doses and Institute (TNAU) – Industry (RSCL) recommended packages.

Key words: Geographic information system (GIS) database, sugarcane, Tamil Nadu, nutrient management.

INTRODUCTION

India is the world's largest producer of sugarcane. Sugarcane is cultivated in about 5.04 million ha producing about 340.56 million tons of cane with an average productivity of 67.57 tonnes/ha (Economic Survey, 2013). The prime concern of cane growers and the sugar industry is to achieve higher sugarcane

support maximum economic returns. The widely varying soil fertility domain is a major limitation for reaching this goal (Mani et al., 2007).

Periyar Vaigai command area of Theni district, Tamil Nadu comprising of about 4,400 ha of sugarcane involving about 4,500 cane fields needs to be spatially and temporally assessed and mapped for its fertility status and to formulate balanced fertilizer recommendation for maximizing sugarcane productivity. The continuous use of micronutrient free high analysis N, P and K fertilizer in the cropping system with the diminishing use of organic manures has resulted in the depletion of micronutrient cations from the soil reserves (Srivastava et al., 2013). Due to lack of knowledge and institutional incapability to implement the developed norms, farmers apply heavy fertilizer doses without considering the current nutrient status of their soils therefore farmers need to be aware of the nature and severity of the nutrient problems in order to arrive at a prudent decision regarding the kind and dose of fertilizer to be applied (Rashid and Ryan, 2004).

In agriculture, global positioning system (GPS) and geographic information system (GIS) technologies have been adopted for better management of land and other resources for sustainable crop production (Palaniswami et al., 2011). Fertilizer is one of the costliest inputs in agriculture and the use of right amount of fertilizer is fundamental for farm profitability and environmental protection (Mahendra, 2010).

The modern day space age technologies can be adopted for speedy dissemination of the research results on optimum doses of nutrients for maximum farm profitability (Singh et al., 2005) to scientists, industry personnel, extension workers and farmers. One of them is the use of soil fertility maps for fertilizer recommendation with a support to calculate fertilizer doses based on soil test values interactively. Inventory of the available macro and micronutrient status of the soils help in demarcating areas where, the application of particular nutrient is needed for profitable crop production (Sood et al., 2009). Assessment and mapping of fertility status of Ghataprabha command area soils which are being intensively cultivated with high yielding crops like sugarcane, maize, wheat and cotton was carried out using GIS (Binita et al., 2009). However detailed characterization and generation of soil fertility maps for the sugarcane predominant and agriculturally significant Theni district of Tamilnadu is virtually lacking and hence present investigation was carried out.

MATERIALS AND METHODS

Soil sampling, processing and analysis

Depending on the variability in physiography, soils and productivity of sugarcane during the previous years, a total of 400 soil samples were collected during the Kharif season of 2011 from the plough layer (0 to 30 cm depth) at an approximate interval of 1 km grid (Figure 1) with the help of hand held global positioning system (GPS) over the major sugarcane growing taluk of Rajshree Sugars and Chemicals Ltd., of Theni district in Tamil Nadu. Each of the taluk maps of Theni district which consist of village boundaries were scanned in tiff format and imported to a GIS system version 9.3. Soil samples were air dried and ground to pass through a 2 mm sieve. The pH and EC was measured with glass electrode in a 1:2.5 soil/water suspension (Jackson, 1973), Organic Carbon estimation by Walkley and Black (1934), available Nitrogen by Subbaiah and Asija (1956), available Phosphorus by Olsen's extractants of 0.5M $NaHCO_3$ as described by Olsen et al. (1954) estimated by Murthy and Riley method using ascorbic acid as reducing agent and as described by Watanabe and Olsen (1965) using spectrometer with red filter at 660 nm wave length.

Available potassium was extracted with neutral 1N NH_4OAc and then measured by flame photometer (Jackson, 1973). The available sulphur in the soil was extracted with 0.15% $CaCl_2.2H_2O$ solution as described by Williams and Steinberg (1959) and estimated by turbidimetric method (Hesse, 1971). The DTPA extractable micronutrients viz., Iron, Manganese, Zinc and Copper was extracted using 1:2 Soil to extractant ratio (Lindsay and Norwell, 1978), and estimated by atomic absorption spectrophotometer model No: ICE 3300 series.

Generation of maps

The Ground Control points were identified for the district and based on them the map was geo-referenced. The revenue village boundaries were digitized in polyconic mode in. *shp* format. After digitization, the necessary corrections were done to clean block and revenue village layer for topology building. The villages were assigned different ids in the layer to assign various attributes in the database. Through Arc catalog software, the columns for available N, P, K and available zinc were added in the layer to enter the attribute data. The available nutrient data were imported from Ms-Excel and assigned to polygon attribute table in the layer. From the attribute database, different thematic layers were reclassified to generate various thematic maps on available nutrients of N, P, K and zinc values. The suitable annotations like legend, palettes, north arrow and scale were composed on thematic maps. The thematic maps of available N, P, K and Zinc of Theni district are thus generated and presented. Based on area the sampling sites were fixed and collected uniformly distributed soils (5-10 Nos.) from each revenue village of the divisions. The samples were analyzed, averaged nutrient status and prepared thematic map by using of Arc GIS 9.3 version.

RESULTS AND DISCUSSION

Physico-chemical properties

The soils of the Theni district as assessed in the major blocks are acidic to alkaline in reaction with pH ranging from 4.70 to 9.2 with a mean value of 7.92. The analytical results of the soil samples collected from 8 divisions showed about 36, 24 and 44% of the soils respectively, in the three division's *viz.,* Jeyamangalam, Theni and Cumbum had a normal pH range of 6.0 to 7.5.

As shown in Table 1, about 52 and 42% of the soils collected from Mayiladumparai division respectively, recorded moderately (7.5-8.5) and highly (>8.5) alkaline pH, while 24% of the soils from Palani Chetti (P.C.) Patti and 28% of Andipatti divisions showed a highly alkaline
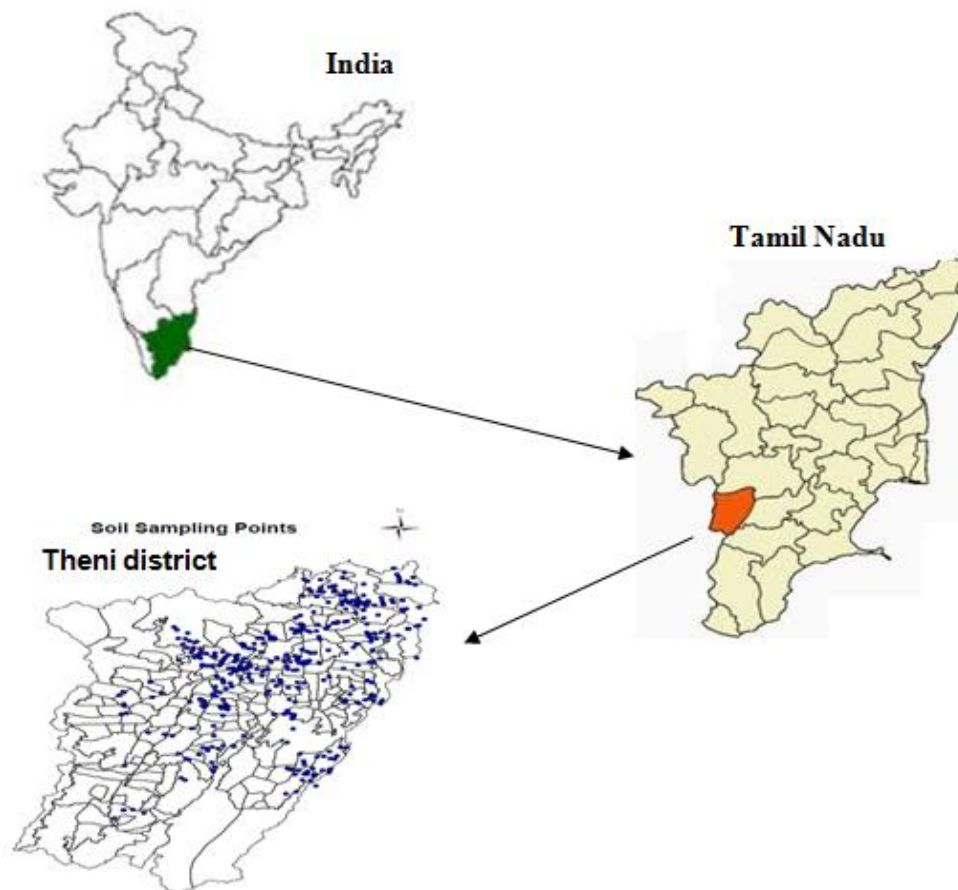
Figure 1. Study area of Theni district of Tamil Nadu.

Table 1. Range of pH and EC status of RSCL sugarcane growing area of Theni district.

| Division | Status (% of samples) | | | | pH | EC (dSm^{-1}) |
	Acidic (<6.0)	Normal (6.0-7.5)	Moderately Alkaline (7.5-8.5)	Highly Alkaline (> 8.5)	Range	Range
Jeyamangalam	-	36	60	4	6.32-8.65	0.05-0.93
Periyakulam	-	18	82	-	6.13-8.45	0.07-0.94
Theni	-	24	76	-	6.78-8.46	0.10-0.64
P.C. Patti	2	18	56	24	5.97-9.11	0.08-0.83
Cumbum	2	44	52	2	4.70-8.60	0.04-1.25
Bodi	2	16	64	18	5.30-8.91	0.04-0.90
Andipatti	-	10	62	28	6.99-9.20	0.03-1.64
Mailadumparai	-	6	52	42	7.32-9.12	0.07-0.89

pH range of 8.5 to 9.2. The variation in pH may be due to the inherent heterogeneity of soils occurrence of various soil types like red, mixed red and black and black soils within the division and to some extent due to influence of parent material and resource region specific differences in the cultural and fertilizer management practices of the cane growers. With the increase of pH the availability of micronutrients decreased, which might be due to precipitation of micronutrients in alkaline soils. This was confirmed by correlation studies that showed the soil pH had significant and negative correlations with available iron (r=-0.5184) and manganese (r=-0.4426) contents.

Table 2. Range and mean value of organic carbon status of RSCL sugarcane growing area of Theni district.

Division	Org. carbon (g kg^{-1})		Status (% of samples)		
	Range	Mean	Low (<0.5%)	Medium (0.5-0.75%)	High (>0.75%)
Jeyamangalam	1.3-13.5	6.6	42	24	34
Periyakulam	0.9-12.1	5.7	36	44	20
Theni	1.3-14.6	5.9	48	36	12
P.C. Patti	0.7-13.6	5.6	48	28	24
Cumbum	0.6-13.9	5.5	46	30	24
Bodi	0.4-12.4	5.1	46	36	18
Andipatti	0.4-11.6	5.0	64	22	14
Mailadumparai	1.2-12.8	5.8	52	26	22

Table 3. Range and Mean value of available nitrogen status of RSCL sugarcane growing area of Theni district.

Division	KMnO$_4$ N (kg ha^{-1})		Status (% of samples)	
	Range	Mean	Low (<280 kg ha^{-1})	Medium (280-450 kg ha^{-1})
Jeyamangalam	154-326	224	86	14
Periyakulam	88-260	200	100	-
Theni	138-367	213	96	4
P.C. Patti	119-279	211	100	-
Cumbum	113-449	225	92	8
Bodi	69-301	187	98	2
Andipatti	97-320	202	96	4
Mailadumparai	91-263	197	100	-

The total soluble salts expressed as electrical conductivity (EC) varied from 0.03 to 1.64 dSm^{-1} in the soils analysed, invariably all the soil samples collected from the 8 divisions of sugarcane growing area recorded EC of less than 2 dSm^{-1} indicating that the soils are free from salinity. As shown in Table 2, the available status of organic carbon ranged from 0.4 to 14.6 g kg^{-1} with a mean value of 5.7 g kg^{-1}. About 36 to 64% of the soils from all the 8 divisions were found to have low organic carbon status (<5.0 mg kg^{-1}). However, about 36% of the soils from Theni and Bodi each were found to have higher organic carbon status.

Available macronutrients

The available nitrogen content of the soil samples varied from 69 to 448 kg ha^{-1} with a mean value of 207 kg ha^{-1} in different revenue villages of the sugarcane growing divisions. As shown in Table 3 and Figure 2, the analytical results of the soil samples showed that almost all the soils from the 8 divisions covering an area of 4224 ha (96%) were low in available nitrogen status (< 280 kg ha^{-1}) based on the critical level of 280 kg ha^{-1} fixed (Muhr et al., 1965). The Cumbum division recorded a wide variation of 113 to 449 kg ha^{-1} of available nitrogen with a mean value of 225 kg ha^{-1}. The low levels may be ascribed to several factors, such as lower organic carbon and high pH. Soil available nitrogen had significant and positive correlation with organic carbon (r=0.3994**) content. These findings are related with those of Sharma and Singh (2001) and Rakesh Kumar et al. (2009).

The available phosphorus content of soil samples varied between 9 and 183 kg ha^{-1} with a mean value of 55 kg ha^{-1}. Among the 400 samples analyzed, about 90 to 95% of the soil samples (3498 ha area) were found to be high in soil available phosphorus status (> 22 kg ha^{-1}) mentioned in Table 4 and Figure 3. The invariably high P status of the soil may be due to continuous application of phosphotic fertilizer (DAP), bio-compost/green plus or rock phosphate enriched compost by the cane farmers of Rajshree sugar mills area as recorded in the farm survey conducted before the preliminary soil sampling study. The application of bio-compost at 3 tonnes ha^{-1} additionally increased 89 kg P$_2$O$_5$ ha^{-1} (1.3% P). Further, the injudicious and long term application of fertilizer by the farmers without assessing the soil available P status might have contributed to soil P buildup. The cane growers of this region adopt the regular practice of soil application of 100 kg FeSO$_4$ and 37.5 kg ZnSO$_4$ ha^{-1}
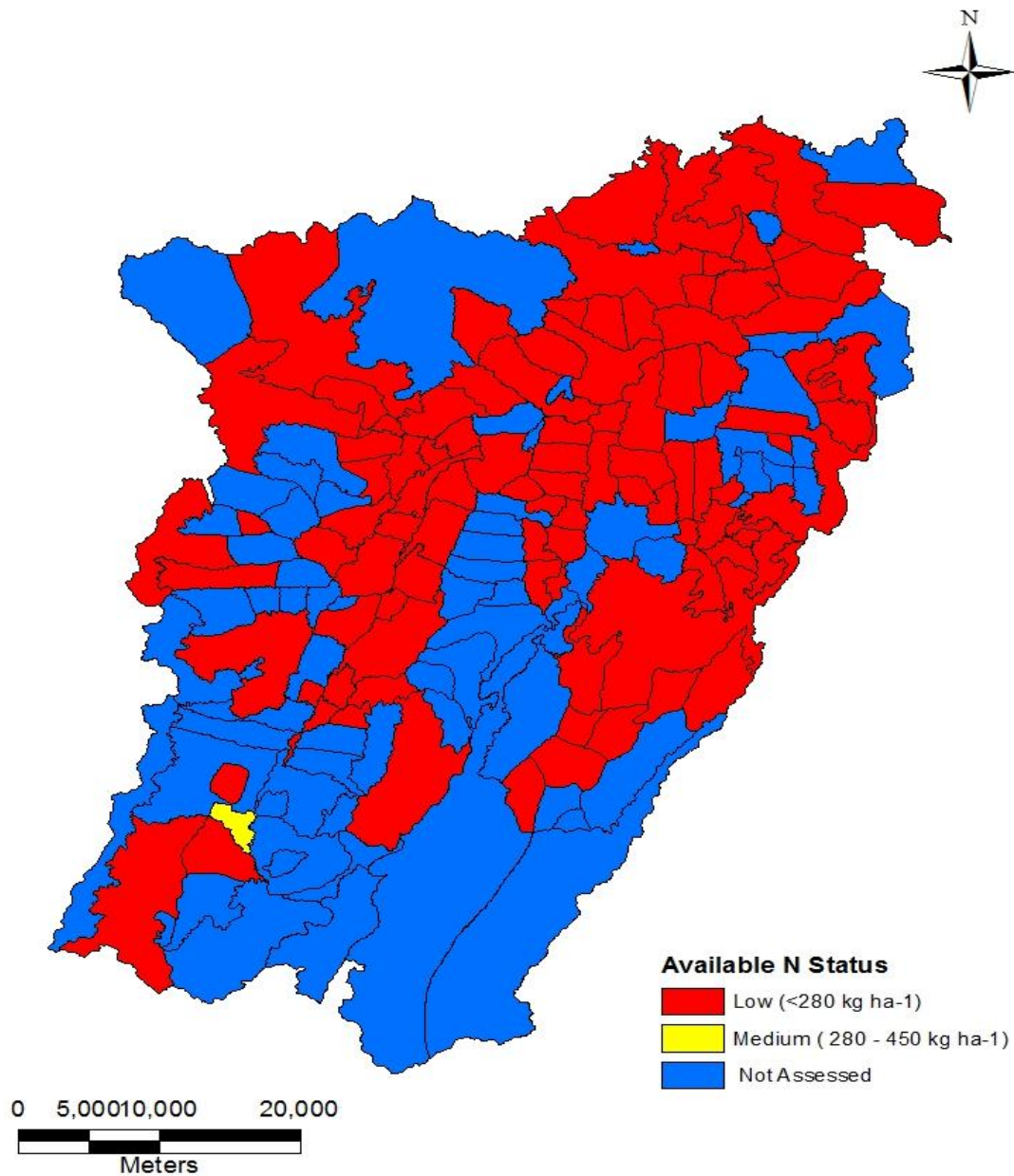
Figure 2. Village level available Nitrogen status of Theni district.

irrespective of the soil Fe and Zn status. However, the available data on DTPA-Zn has indicated Zn deficiency in more than 50% soil samples of the 6 out of the 8 divisions analyzed highly the antagonistic relationship between phosphorus and zinc. Thus, there is an indication of build-up of phosphorus in sugarcane growing areas of Theni district under high intensity irrigated cultivation. Bali et al. (2010) also reported a similar trend in soils of Punjab where, about 45% of the area is high in available P status.

The available potassium content of soil samples ranged from 83 to 2010 kg ha^{-1} with a mean value of 514 kg ha^{-1}.

As shown in Figure 4, about 20 to 35% of the soils from these areas recorded medium status of available potassium. The highest mean value of available K (636 kg ha^{-1}) was recorded in P. C. Patti and the lowest mean value of 203 kg ha^{-1} was recorded in Andipatti division of Theni district (Table 5). Available potassium had a significant positive correlation with EC (r=0.2976*), OC (r=0.2204*) and available nitrogen (r=0.2491*) which was in alignment with the results reported by Bali et al. (2010). The average available sulphur content in the soil samples varied from 1.02 to 98 ppm with a mean value of 20 ppm. Considering 10 ppm as critical limit for available sulphur,

Table 4. Range and mean value of available phosphorus status of RSCL sugarcane growing area of Theni district.

Division	Olsen P (kg ha^{-1})		Status (% of samples)	
	Range	Mean	Medium (11-22 kg ha^{-1})	High (>22 kg ha^{-1})
Jeyamangalam	20-283	55	4	96
Periyakulam	9-127	45	18	80
Theni	31-135	69	-	100
P.C. Patti	23-142	72	-	100
Cumbum	9-131	51	14	84
Bodi	24-135	58	4	96
Andipatti	11-142	50	20	80
Mailadumparai	11-90	42	34	66
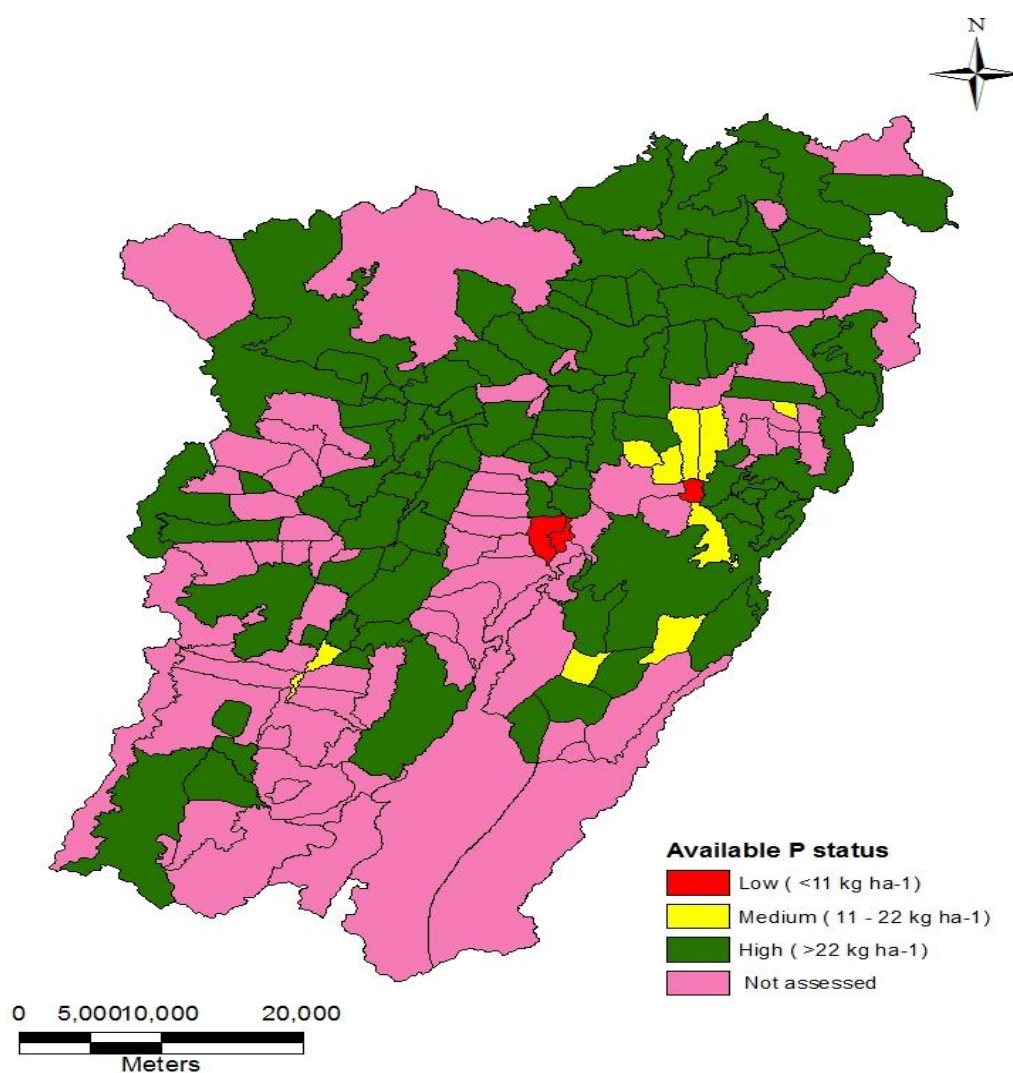
Figure 3. Village level available phosphorus status of Theni district.

50% of soils of the sugarcane growing area are found to be adequate in sulphur content. The average calcium carbonate content in the soil samples varied from 1.0 to 47.3 g kg^{-1} with a mean value of 12.2 g kg^{-1} in all the
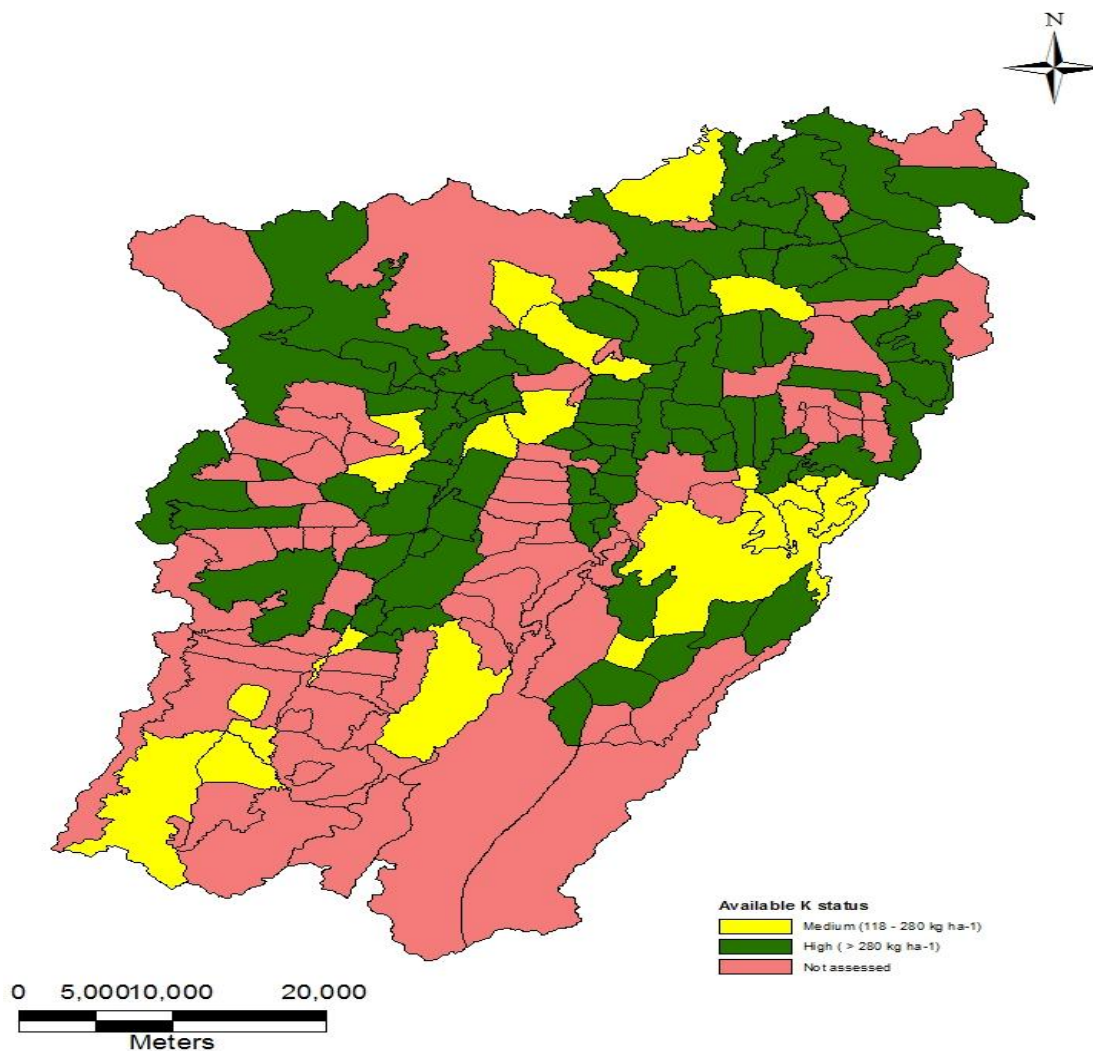
Figure 4. Village level available potassium status of Theni district.

Table 5. Range and mean value of available potassium status of RSCL sugarcane growing area of Theni district.

Division	Neutral N K (kg ha^{-1})		Status (% of samples)		
	Range	Mean	Low (<118 kg ha^{-1})	Medium (118- 280 kg ha^{-1})	High (>280 kg ha^{-1})
Jeyamangalam	130-1518	596	-	20	80
Periyakulam	93-1501	354	2	36	62
Theni	140-1820	564	-	18	88
P.C. Patti	85-2010	636	4	22	74
Cumbum	152-1696	523	-	32	68
Bodi	83-1613	440	4	32	64
Andipatti	130-1098	536		24	76
Mailadumparai	94-1714	460	2	16	82

divisions. Invariably all the soil samples collected from the 8 divisions of sugarcane growing area recorded CaCO$_3$ of less than 50 g kg^{-1} (critical limit for free CaCO$_3$) indicating that the soils are free from calcareousness.

Table 6. Range of available iron and manganese status of RSCL sugarcane growing area of Theni district.

Division	Fe (ppm)	Status (% of samples) (Critical limit 3.7)		Mn (ppm)	Status (% of samples) (Critical limit 2.0)	
	Range	Deficient	Sufficient	Range	Deficient	Sufficient
Jeyamangalam	2.0-82.4	10	90	2.1-38.4	-	100
Periyakulam	2.5-91.9	8	92	1.0-21.4	2	98
Theni	1.4-18.1	18	82	1.2-18.2	30	70
P.C. Patti	1.8-128.6	22	78	2.1-17.1	-	100
Cumbum	3.3-128.9	4	96	2.1-37.3	-	100
Bodi	1.8-84.6	12	88	2.4-38.5	-	100
Andipatti	2.0-138.0	24	76	2.4-45.1	-	100
Mailadumparai	2.2-72.1	46	54	2.2-18.7	-	100

However, the available rate of calcium carbonate content had a significant and positive correlation with soil pH (r=0.3358**) and a non-significant negative correlation with available iron (r=-0.1505).

Available micronutrients

The available iron content of soil samples varied from 1.4 to 129 mg kg^{-1} with a mean value of 12.99 mg kg^{-1} and is comparable with those reported by Talukdar et al. (2009) in soils of Assam. The highest mean value was found in Cumbum division (21.28 mg kg^{-1}) and lowest was found in Mailadumparai division (7.51 mg kg^{-1}). About 54 to 96% of samples from five divisions were found to have available iron content above the critical limit (3.7 mg Fe kg^{-1}). However from Mailadumparai, Andipatti and P.C. Patti divisions had 46, 24 and 22% of soil samples were deficient in available Fe. This might be due to precipitation of Fe^{2+} in higher pH of the sodic soils in these soils and this can be confirmed through the significant and negative correlation of soil pH with available iron (r=-0.5184**) which was also reported by Verma et al. (2007).

As shown in Table 6, the available manganese content of soil samples ranged from 0.98 to 45.10 mg kg^{-1} with a mean value of 7.76 mg kg^{-1}. The highest and lowest mean values 13.37 and 3.73 mg kg^{-1} respectively of available Mn content were recorded in Cumbum and Theni division. All the divisions recorded sufficient available Mn content except Theni division, where 30% of the samples were observed to be deficient in DTPA-Mn. The Mn bearing minerals in the parent material of these soils might be the reason for higher Mn content of soils. Negative significant correlations of available Mn with pH (r=-0.4426**) and non-significant correlation with organic carbon (r=0.0506) was also reported. Similar results were also reported in Lachimpur series of Jharkhand (Rakesh et al., 2009) which may be due to the formation of

insoluble higher valent oxides of Mn at high pH (Naheed et al., 2010).

The available zinc content of soil samples ranged from 0.1 to 12.78 mg kg^{-1} with a mean value of 1.60 mg kg^{-1}. As shown in Figure 5, more than 50% of soil samples were found to be deficient from all divisions except Jeyamangalam, Cumbum and Mailadumparai where, 80, 66 and 56% of samples, respectively were observed to be sufficient in available zinc (1.2 mg kg^{-1} critical limit) as per Muhr et al. (1965). The higher sufficient content of available zinc might be due to highest content of organic carbon as well as finer fractions of soils leading to increase in the surface ion exchange and hence contributed to the higher amount of DTPA-Zn in those soils (Sharma et al., 2003). This was confirmed when the available zinc content of soil samples showed a significant and positive correlation with organic carbon (r=0.277*) mentioned in Table 7. A significant and negative correlation (r=-0.3050*) (Table 8) with pH indicated the availability of Zn decreases with increase in soil pH.

The available copper content of soil samples from all the cane divisions of sugar mill ranged from 0.52 to 11.13 mg kg^{-1} with a mean value of 2.42 mg kg^{-1}. All the divisions recorded sufficient in status of available copper content. It showed a significant and positive correlation with organic carbon, available nitrogen, phosphorus and potassium, DTPA-Fe and Zn Table 7. The increased availability of Cu^{2+} may be due to the fact that, DTPA being organic chelating agent extracts micronutrient cations from different pools and the higher amount of organic carbon coupled with low pH values is further likely to increase the solubility of micronutrient cations (Talukdar et al., 2009).

Conclusions

It can be concluded that the soils of the Theni district as

Theni
Available Zn Status

- Not assessed
- Deficient (<1.2 ppm)
- Sufficient (>1.2 ppm)
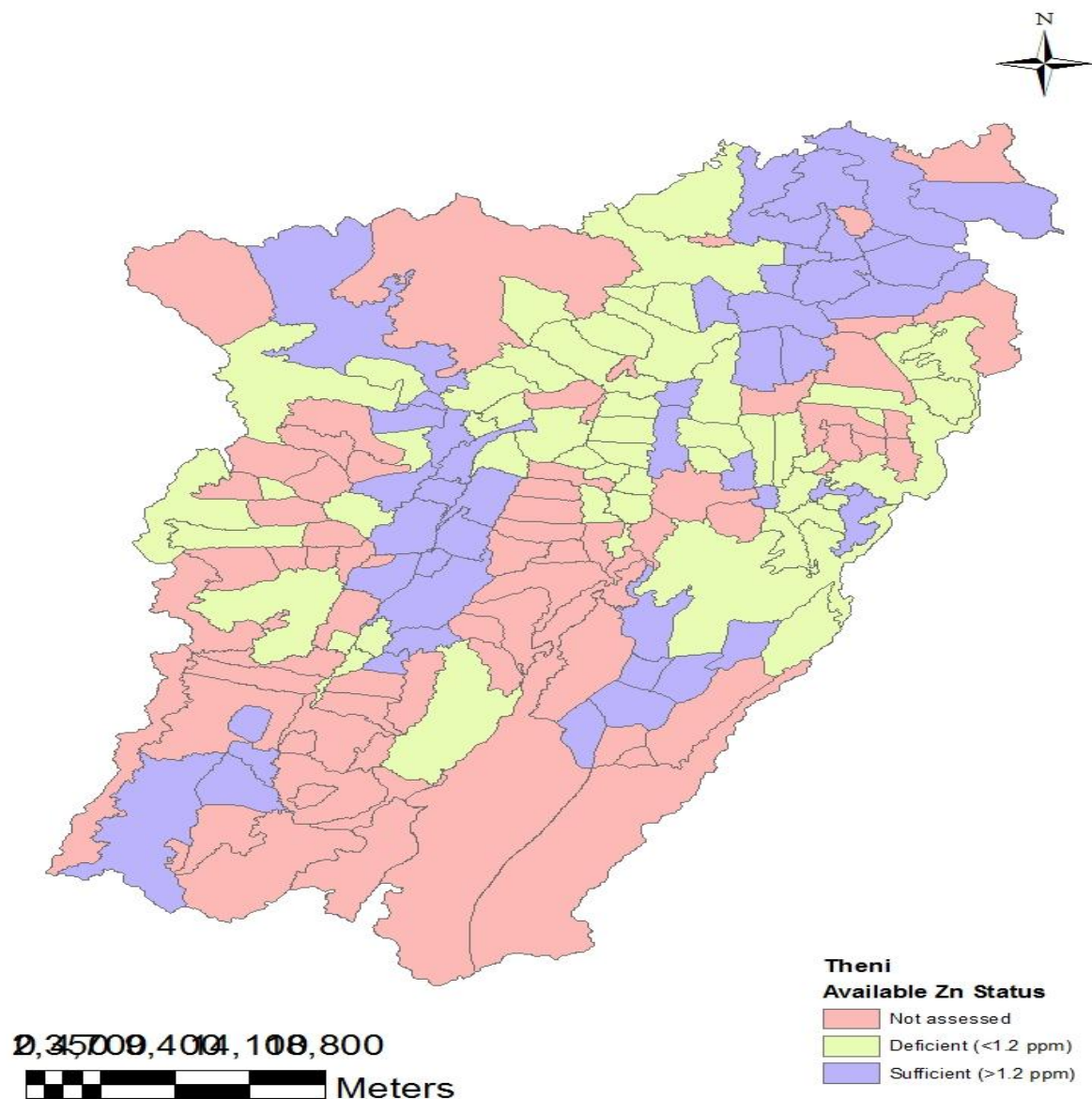
Figure 5. Village level available Zinc status of Theni district.

Table 7. Range of available zinc and copper status of RSCL sugarcane growing area of Theni district.

Division	Zn (ppm)	Status (% of samples) (Critical limit 1.2 ppm)		Cu (ppm)	Status (% of samples) (Critical limit 1.2 ppm)	
	Range	Deficient	Sufficient	Range	Deficient	Sufficient
Jeyamangalam	0.4-9.9	20	80	1.0-11.1	4	96
Periyakulam	0.3-9.7	62	38	1.3-4.8	-	100
Theni	0.2-3.4	74	26	0.9-7.8	8	92
P.C. Patti	0.1-7.7	58	42	0.8-8.2	8	92
Cumbum	0.2-12.8	34	66	0.8-7.3	10	90
Bodi	0.2-8.3	66	34	0.7-5.5	18	82
Andipatti	0.2-5.3	56	44	0.5-3.9	24	76
Mailadumparai	0.2-6.7	44	56	0.6-2.9	22	78

Table 8. Correlation between soil physico-chemical properties and available nutrients of surface soils.

	pH	EC	OC	Av. N	Av. P	Av. K	CaCO$_3$	Av. S	Av. Fe	Av. Mn	Av. Zn	Av. Cu
pH	1.0	0.12	-0.230*	-0.172	-0.024	0.154	0.336*	0.035	-0.518**	-0.443*	-0.305*	-0.302*
EC		1.00	0.204*	0.103	0.097	0.298*	0.031	0.561**	-0.172	-0.025	-0.001	0.049
OC			1.00	0.399**	0.238*	0.220*	-0.016	0.207*	0.148	0.051	0.277*	0.367**
Av. N				1.00	0.165	0.249*	0.007	0.059	0.050	0.002	0.061	0.328**
Av. P					1.00	0.197	-0.123	0.040	-0.270*	-0.082	-0.235*	0.110
Av. K						1.00	0.069	0.276*	-0.184	-0.086	0.018	0.025
CaCO$_3$							1.00	0.007	-0.151	-0.102	-0.070	-0.173
Av. S								1.00	-0.035	0.014	0.024	-0.011
Av. Fe									1.00	0.357***	0.438**	0.290*
Av. Mn										1.00	0.18029	-0.049
Av. Zn											1.00	0.186
Av. Cu												1.00

assessed in the major blocks are acidic to alkaline in reaction. Invariably all the soil samples collected from the 8 divisions of sugarcane growing area recorded EC of less than 2 dS m^{-1} indicating that the soils are free from salinity. Low available nitrogen status (< 280 kg ha^{-1}) indicates that nitrogen is the major limiting nutrient in the soils. The soil samples were high in available phosphorus (> 22 kg ha^{-1}) and high in available potassium (>280 kg ha^{-1}) status. More than 50% of the soil samples were deficient in available zinc from all the sugarcane growing divisions except Jeyamangalam. The maps generated under the study will be useful for generating homogenous units and guiding the farmers to decide the amount and kind of macro as well as micronutrients to be applied for optimizing/economic returns based on the site specific nutrient management. The geo-referenced sampling sites can be revisited with the help of GPS, which helps in monitoring the changes in the status of nutrients over a period of time, which otherwise is not possible by traditional methods of sampling.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Effect of date of sowing and crop geometry on growth and yield parameters of forage mustard (Var. *Chinese Cabbage*)

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A field experiment was conducted during *rabi* 2011-2012 at Forage Management and Research Centre, National Dairy Research Institute, Karnal to study the "Effect of date of sowing and crop geometry on seed yield and quality of forage mustard (var. Chinese cabbage)". The soil texture of the experimental site was clay loam with available nitrogen, phosphorus, potassium were 212, 20 and 256 kg/ha, respectively. There were 24 treatment combinations consisting of four dates of sowing (1st October, 16th October, 31st October and 15th November) and six crop geometry (60×15, 45×15, 45×20, 45×25, 30×20 and 30×25 cm) and the experiment was laid out in split plot design with four replications. From the result, sowing during 1st October recorded significantly higher crop yield attributing characters viz. no. of primary, secondary and tertiary branches, number and weight of siliquae /plant and ultimately seed yield (2013 kg/ha) with better growth and higher dry matter accumulation in yield components compared to 31st October and 15th November. Crop geometry of 30×20 cm recorded significantly higher seed yield (1829 kg/ha) with better utilization of space, nutrients, water and sunshine resulting in higher dry matter translocation to yield components as compared to 60×15, 45×15, 45×20, 45×25 and 30×25 cm crop geometry.

Key word: Crop geometry, date of sowing, forage mustard, seed yield.

INTRODUCTION

Mustard [*Brassica juncea* L.], an important edible oil seed crop of India belongs to family Brassicaceae. It is known to Greeks, Romans, Indians and Chinese 2000 years ago. Genus *Brassica* comprises of five cultivated species *viz., Brassica juncea* (Indian mustard), *Brassica campestris* (Toria), *Brassica nigra* (Banarasi rye), *Brassica napus* (Gobhi sarson) and *Brassica carinata*

(Abyesinian mustard) predominantly grown in China, India ,Canada, Pakistan, USSR and Europe. Among these, *Brassica compestris* (Toria) var. Chinese cabbage is grown as fodder crop in India. Rapeseed mustard crop in India is grown in diverse agro climatic conditions ranging from northeastern / north western hills to down south under irrigated/rainfed, timely/late sown, saline

soils and mixed cropping. In India, Chara Sarson (Chinese cabbage) occupies 10,000 ha area and productivity is 15 to 35 tonnes/ha green fodder (Handbook of Agriculture, 2010). Rapeseed-mustard follows C_3 pathway for carbon assimilation. Therefore, it has efficient photosynthetic response at 15 to 20°C temperature. At this temperature the plant achieve maximum CO_2 exchange range which declines thereafter (Singh et al., 2001). Mustard oilseed cake is used as livestock feed and green leaves and stem are used as a green fodder for livestock. The competitive ability of a rapeseed- mustard plant depends greatly upon the density of plants per unit area and soil fertility status. The optimum plant population density/unit area varies with the environment, the genotype, the seeding time and the season. Uniform distribution of crop plants over an area results in efficient use of nutrients, moisture and suppression of weeds leading to high yield (Sonani et al., 2002). Mustard sown on 14th and 21st October took significantly more days to 50% flowering (55 and 57) and maturity (154 and 156) compared to 7th October planting (Kumar et al., 2001). Delayed sowing resulted in poor growth, low yield and oil content. Date of sowing influence the incidence of insect-pest and disease also. Sowing on 21st October resulted in least *Sclerotina* incidence (Gupta et al., 2004). So in this backdrop the present investigation was undertaken to study the effect of date of sowing on growth and seed yield of forage mustard (var. Chinese cabbage).

MATERIALS AND METHODS

A field experiment was conducted at Forage Research and Management Centre (FRMC), National Dairy Research Institute, Karnal, India during *rabi* 2011-2012. The soil was clay loam in texture, low in available N, medium in organic carbon, available P, and available K. The experimental treatment consist of 4 date of sowing (1[st]Oct.,16th Oct.,30st Oct.,15th Nov.) and 6 crop geometry (60×15, 45×15, 45×20, 45×25, 30×20, 30×25 cm). The experiment was laid out in a split plot design with 24 treatment combinations with four replications. The forage mustard was raised by following recommended agronomic cultural practices. The growth and yield parameters were taken for at different date of sowing. During October 2011 to March 2012, mean weekly maximum temperature ranged from 33.1°C (10-16th October) to 15.5°C (16-22nd January), while the mean weekly minimum temperature ranged from 20.8°C (3-9th October) to 3.8°C (19-25th December).

RESULTS AND DISCUSSION

Date of sowing as well as crop geometry affected yield and yield parameters in forage mustard (Var. Chinese Cabbage). No. of siliquae/plant, weight of siliquae/plant significantly affected Sowing during 1st October (1391.47/plant) recorded significantly higher No. of siliquae/plant as compared to 16th October (1227.96/plant) and, 31st October (847.41/plant) and 15th November (656.75/plant) sowing, respectively (Table 1).

These were mainly due to favorable temperature and nutrient dynamics (Dotaniya and Meena, 2013) enhanced higher number of primary, secondary and tertiary branches at harvest (Kurmi, 2002). Whereas crop geometry of 60×15 cm (1526.52/plant) recorded significantly higher number of siliquae/plant as compared to 45×15 cm (906.58/plant), 45×20 cm (988.90/plant), 45×25 cm (1291.41/plant), 30×20 cm (745.76/plant) and 30×25 cm (727.21/plant), respectively. This was mainly due to wider spacing less competition for space and nutrients. Increasing spacing enhanced the root extension and better uptake of nutrients by crop roots (Singh et al., 2001, 2002; Shivani and Kumar, 2002). The weight of siliqua/plant and weight of siliqua/m^2 was found significantly with respect to dates of sowing and crop geometry. Sowing during 1st Oct. significantly higher value in (D1)> (D2)> (D3) > (D4). These results are in conformity with results of Panda et al. (2004).

Stover yield was found significant with respect to dates of sowing and crop geometry. Stover yield remained at par between 1st and 16th October sowings and recorded significantly higher yield as compared to 31st October (D3) (6217.20 kg/ha) and 15th November (D4) (4774.3 kg/ha) sowing, respectively. Whereas, crop geometry of 30×20 cm (8125 kg/ha) recorded significantly higher Stover yield as compared to 45×20 cm (7418.98 kg/ha), 45×25 cm (7410.3 kg/ha) and 30×25 cm (8043.98 kg/ha), respectively. It may be due to higher plant height at 30, 60 DAS and at harvest, number of primary, secondary and tertiary branches. Singh and Singh (2002) also reported higher Stover yield in the early sowings. It might be due to higher GDD, PTU, and day taken to attain physiological maturity stage in these sowing dates. The detrimental effect of heat at a later stage of crop development and earing in delayed sowing had an adverse effect on grain yield (Amrawat et al., 2014). This was mainly due to higher plant density. These results are in conformity with the results of Kumar et al. (1997).

The Stover seed yield was found significant with respect to dates of sowing and crop geometry. Sowing during on (D1) recorded significantly higher grain yield as compared to (D3), (D4). There was 18% increase in seed yield with 1st October sowing over 31st October sowing. The decrease in seed yield with delay in sowing occurred coupled with primarily due to poor dry matter built up which led to reduced bearing capacity. Slower growth at low temperature during early vegetative growth phase and the overall shorter crop duration. Reduced seed-filling duration caused by forced maturity because of sudden rise in temperature during maturity phase, resulted in poor sink strength (Angrej et al., 2002). Crop geometry of 30×20 cm (1829.16 kg/ha) recorded significantly higher seed yield as compared to 60×15 cm (1504.65 kg/ha), 45×15 cm (1640.28 kg/ha) and 45×20 cm (1659.43 kg/ha) spacing, respectively. Wider spacing could not fully utilized the available soil nutrients, moisture and light consequently reducing the seed yield

Table 1. Effect of date of sowing and crop geometry on crop growth and yield parameters.

Treatments		No. of siliquae/ plant	wt. of siliquae/plant (g)	wt. of siliquae/m^2 (g)	Stover yield (kg/ha)	Seed yield (kg/ha)
Date of sowing						
D1	1st Oct.	1391.47	96.89	988.50	10021.21	2013.48
D2	16th Oct.	1227.96	86.08	889.46	10147.57	1837.96
D3	31st Oct.	847.41	64.66	659.71	6217.20	1649.69
D4	15th Nov.	656.75	43.12	467.45	4774.30	1216.62
CD (5%)		16.74	10.41	106.01	318.48	240.09
Crop geometry (cm)						
S1	60x15	1526.52	97.63	785.80	8000.58	1504.65
S2	45x15	906.58	65.96	806.16	7741.60	1640.28
S3	45x20	988.90	71.27	689.93	7418.98	1659.43
S4	45x25	1291.41	89.32	677.00	7410.30	1706.36
S5	30x20	745.76	56.71	848.64	8125.00	1829.16
S6	30x25	727.21	55.24	700.16	8043.98	1736.74
CD (5%)		25.94	8.18	91.98	266.98	146.09

(Momoh et al., 2004). Increased competition in higher density plants caused reduction in the number of pods on all branches. However high density resulted in higher yield compared to wider spacing whereas per plant branches and no. of pods were high. The results are in conformity with the findings of Sahoo et al. (2000) and Chaniyara et al. (2002).

Conclusion

In conclusion, sowing of forage mustard as early as in the present study (1st October) seems to be a feasible strategy for increasing yield and controlling weeds and conservation of soil moisture and, better nutrient dynamics during crop growth. Crop geometry affected the crop physiology and yield parameters. Ideal spacing (30×25 cm) provide better aeration, soil moisture, plant nutrient and improved the microclimate for crop growth and ultimately seed yield.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Effect of defoliation from the bottom to the top on the yield of Brazilian soybean cultivars

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Among the factors that may cause decreases in soybean yield there are the foliar diseases that despite control it can limit grain yield. The Asian soybean rust (*Phakopsora pachyrhizi*) is considered the most important disease of soybean in Brazil. The aim of this work was to simulate the progress of Asian rust in Brazilian soybean cultivars by removal of trifoliate leaves from the bottom to the top. The experimental design was a randomized complete block, in a 3x3x5 factorial scheme, with four replications. It was observed that there was significant interaction between cultivars and defoliation for all yield components and consequently in seed yield. Increase in the defoliation intensity towards the bottom to the top during the reproductive stages studied (R3, R5, R6) there is a linear decrease in grain productivity, reaching in the highest defoliation level, loss of 79.6, 77.7 and 38.6% in R3, R5 and R6 stages, respectively. The simulation of damage by soybean rust through defoliation cultivars showed severity in leaf area reduction and its consequent effect in grain seed yield.

Key words: Yield components, leaf area, reproductive stage, biotic stress.

INTRODUCTION

In Brazil, soybean has the highest seed yield, and in the season 2012/2013, the average yield was 2938 kg ha^{-1} (CONAB, 2014). Among the factors that could cause losses to this crop, there are the foliar diseases that despite control management can limit yield. Asian rust (*Phakopsora pachyrhizi*) is considered the most significant disease of soybean in Brazil. Its biggest loss is caused by the premature abscission of leaves and their higher incidence and severity is mainly in the reproductive stages of the crop.

The soybean rust was first recorded in Brazil in 2000/2001 season and from there it spread throughout the country. In 2004, the losses caused by the disease (sum of seed losses, control expenses and reduced government revenues) were of US$ 2.28 billion (Yorinori and Lazzarotto, 2004). In more severe cases, without proper control, soybean rust can cause yield losses of about a 100% (Navarini et al., 2007; Oliveira, 2004; Barros et al., 2008; Yorinori, 2002). The temperature ideal for disease development is 15 to 28°C, with 6-12

Table 1. Agronomic characteristics of Brazilian soybean cultivars.

Cultivar	Growth type	Maturity group	Shape leaflet
M 7211 RR[1]	Indeterminated	7.2	Pointed oval
TMG 1176 RR[1]	Determinated	7.6	Lanceolate
M 7188 RR[1]	Determinated	8.8	Oval

[1]Commercial name.

hours of moisture on the leaf needed for spore germination (Dorrance et al., 2007). The disease starts from the bottom leaves where there is more moisture after the closure of plants in the area, providing favorable conditions. Without control, the disease progresses to the upper leaves and consequently accelerates abscission of leaves and reduces the effective leaf area of the plant.

Understanding the physiology of soybean production is important to understand the disease effect on yield. The yield is defined as a function of radiation absorbed by the crop canopy (leaves), the conversion of solar radiation absorbed by the plant in dry matter (that is, the efficiency use of the radiation) and the proportion of total plant dry matter accumulated during the growth period that is allocated to the seed (harvest index) (Hay and Porter, 2006). The main soybean yield components are the number of pods/plant, number of seed/plant (product of the number of pods x number of seeds/pod) and the seed weight. Understanding the influence of each component in yield may reveal answers on how to improve the yield in this legume.

The early defoliation in soybeans causes yield loss by interference in physiological processes such as photosynthesis, resulting in fewer pods, fewer seeds per plant, seeds viable per pod and lower seed weight (Ribeiro and Costa, 2000). Artificial defoliation made between R5 and R6 stages showed lower seed filling (Peluzío et al., 2002) and leaf removal in R4 stage caused yield loss of up to 93.4% (Barros et al., 2002; Peluzío et al., 2002). At R3 and R4 stages, the defoliation causes pods abortion at a time when the plant has peak photosynthetic activity for forming and filling it.

The quantification of defoliation can be used as a parameter for estimating damage, to evaluate treatments for disease control and tests of genotype to resistance to Asian rust as well (Hirano et al., 2010). Researches aimed at quantification and progress of Asian rust in soybean is important to establish better control strategies. The study of damage levels of soybean rust can be simulated through artificial defoliation of plants from the bottom to the top.

Most papers of defoliate simulation in soybean were conducted to simulate the attack by insects (Bahry et al., 2013; Bueno et al., 2010; Gregorutti et al., 2012; Timisina et al., 2007; Fontoura et al., 2006) being few studies that were performed to simulate defoliation diseases (Aqeel,

2011). Thus, the objective of this research was to simulate the progress of Asian soybean rust cultivars by removal of trifoliate leaves from the bottom to the top.

MATERIALS AND METHODS

The trial was conducted in a greenhouse at the Universidade Federal de Viçosa, Minas Gerais State, Brazil. The soil had the following chemical characteristics: pH (H_2O) = 5.06; P (Mehlich 1) = 2.2 mg dm^{-3}; K = 32 mg dm^{-3}; Ca = 1.28 $cmol_c$ dm^{-3}; Mg = 0.38 $cmol_c$ dm-3; Al = 0.49 $cmol_c$ dm^{-3}; H + Al = 5.80 $cmol_c$ dm^{-3}; Organic matter = 2.94 g dm^{-3}; CTC (pH 7.0) = 7.54 $cmol_c$ dm^{-3}; Base saturation (V%) = 23.1. It also had a clay texture. Based on these results were applied calcitic lime (1 g kg^{-1} of soil) and to fertilizer was used 300 and 150 mg kg^{-1} of soil of P and K, respectively.

The experimental design was a randomized complete block. Treatments resulted from the combination of three factors (3x5x3), with four replications. Factors consisted of defoliation stage (R3, R5 and R6), defoliation levels (no defoliation, 2, 4, 6 and 8 trifoliate leaves from the bottom to the top) and cultivars (TMG 1176 RR, M 7211 RR and TMG 7188 RR). Each plot consisted of a pot of 2.5 L of soil with two plants. The stages for performing defoliation were considered according to classification of Fehr and Caviness (1977):
R3: had pod with 5 mm length in one of the last four upper nodes on the main stem with a fully developed leaf.
R5: had seed with 3 mm length in a pod located in one of the last four upper nodes on the main stem with a fully developed leaf.
R6: had pod containing green seed that fills the pod cavity located in one of the last four upper nodes on the main stem with a fully developed leaf.

The defoliation levels studied were considered according to the disease in the field, because without proper control it progresses rapidly to the upper leaves. The goal of each level was to simulate that disease would be controlled at that particular point without progress to the upper leaves.

The available cultivars have different characteristics in relation to maturity group; growth type and leaf shape (Table 1). This contrast, allows us to have more reliable results because the genetic variability within Brazilian soybean cultivars was considered.

The defoliation was artificially performed with scissors in their respective stages, removing the trifoliate leaves and keeping the petiole, as well as in disease occurrence in the field, where the leaflets first become detached from the petiole. In the case of the cultivar M 7211 RR, which had an indeterminate growth, with the removal of trefoil in R3, the plants did not have the largest number of trifoliate leaves of treatment (8 trifoliate), it was removed as soon as the next developed.

The sowing was carried out using five seeds/pot and at V1 stage. Thinning was done by keeping the two most vigorous plants. Number of pods/plant, number of seeds/plant, 100-seed weight and seed yield/plant were evaluated. The data were subjected to variance and regression analyses and mean comparisons in Genes software (Cruz, 2013).

Table 2. Summary of variance analysis for number of pods/plant (NPP), number of seeds/plant (NSP), 100-seed weight (100SW) and seed yield/plant (SYP).

Variation source	df	Means square			
		NPP	NSP	100SW	SYP
Block	3	5,078	34,8051	10,8745	0,5316
Cultivar (C)	2	1226,2764**	6945,6681**	505,9898**	321,7979**
Stage (S)	2	675,1847**	3357,3347**	26,9012**	35,5611**
Defoliation (D)	4	632,7861**	2929,0681**	185,6922**	163,825**
C x S	4	11,416	25,416	5,3386	0,6902
C x D	8	21,0819*	87,8191**	8,0205*	8,1668**
S x D	8	108,9174**	648,2378**	2,6732	8,912**
C x S x D	16	10,7944	48,1295	1,9961	1,6597
Error	132	8,5121	32,4689	2,4918	0,9901
Mean		15,75	34,13	15,46	15,51
Coefficient of variation (%)		18,52	16,69	10,20	18,04

* Significant at 5% probability by F test, ** Significant at 1% probability by F test.

RESULTS

In variance analysis (Table 2), it was observed that there was significant interaction between cultivars and defoliation levels for all yield components and consequently in the productivity. The interaction between defoliation stages time and defoliation levels only was not significant for 100-seed weight, which component had isolated influence of defoliation stage.

Regarding the number of pods/plant (Table 3), it was observed that within all removal levels of trifoliate leaves, the TMG 7188 RR cultivar was outstanding. The M 7211 RR produced significant pod number smaller, regardless of the number of leaves removed. Through regression analysis, it was noted that all cultivars had a linear decrease in the pod number according to increase in the number of trifoliate leaves removed (Figure 1A).

Regarding the number of seeds/plant (Table 3), the cultivar M 7211 RR produced significantly lower number than the others. This performance is due to the reduced pod number combined with low number of seeds per plant (approximately 1.80) produced by this cultivar. At all defoliation stages there was a linear decrease in the number of seeds/plant (Figure 1B). Differently the number of pods/plant, the number of seeds/plant produced by TMG 7188 RR cultivar was significantly higher. The number of seeds produced by TMG 7188 RR was very close to the TMG 1176 RR, because this last cultivar has the greater number of seeds/pod trait that is genetically linked to the lanceolate leaf character.

Comparing the 100-seed weight of cultivars within the levels of defoliation (Table 3) showed that the TMG 7188 RR cultivar had higher seed weight in comparison with the others, regardless of how many trifolioliate leaves was removed. In the M 7211 RR cultivar from the removal of four trifoliate had larger reductions in seed weight.

The three cultivars had a significant linear decrease in seed weight with the increase in defoliation (Figure 1C). The decrease in this component is smaller than the others, in which case the decrease is mainly caused by leaf removal in R5, a critical moment the plant should not go through stress, because the number of seeds was already defined and it is starting filling.

The seed yield was significantly different within all defoliation levels performed (Table 3). Until removal of four trifoliolate, the productivity remained the order of TMG 7188 RR> TMG 1176 RR> M 7211 RR. With the removal of 6 and 8 trefoils the TMG 7188 RR and TMG 1176 RR not statistically different, suggesting that this cultivar, even being of lower maturity group (7.6) can maintain certain yield in higher defoliation, when compared to maturity group 8.8. For yield in relation of trifoliolate leaves removed (Figure 1D), as well as all yield components, there was a linear decrease for all cultivars and despite the difference in the yield, the trend was similar for all.

To the number of pods on each level at different defoliation stages (Table 4), it was observed that after the removal of the two first trifoliate leaves, this yield component began to be affected in R3 and R5 stages, when compared to R6 stage, wherein the defoliation no more affects this component. This result can be seen best in Figure 2, which was found a linear decrease for the defoliation at R3 and R5 stages, reaching more than 50% of reduction in the number of pods with the removal of 8 trefoils.

The influence of trifolioliate leaves removal at different stages in the number of seeds/plant can be seen in Table 4, where it was found the same behavior of the number of pods/plant. From the removal of two trefoils in R3 and R5 stages there was significant decrease in the number of seeds/plant, compared with defoliation in the R6 stage, which there is no more change to this trait. This is due to
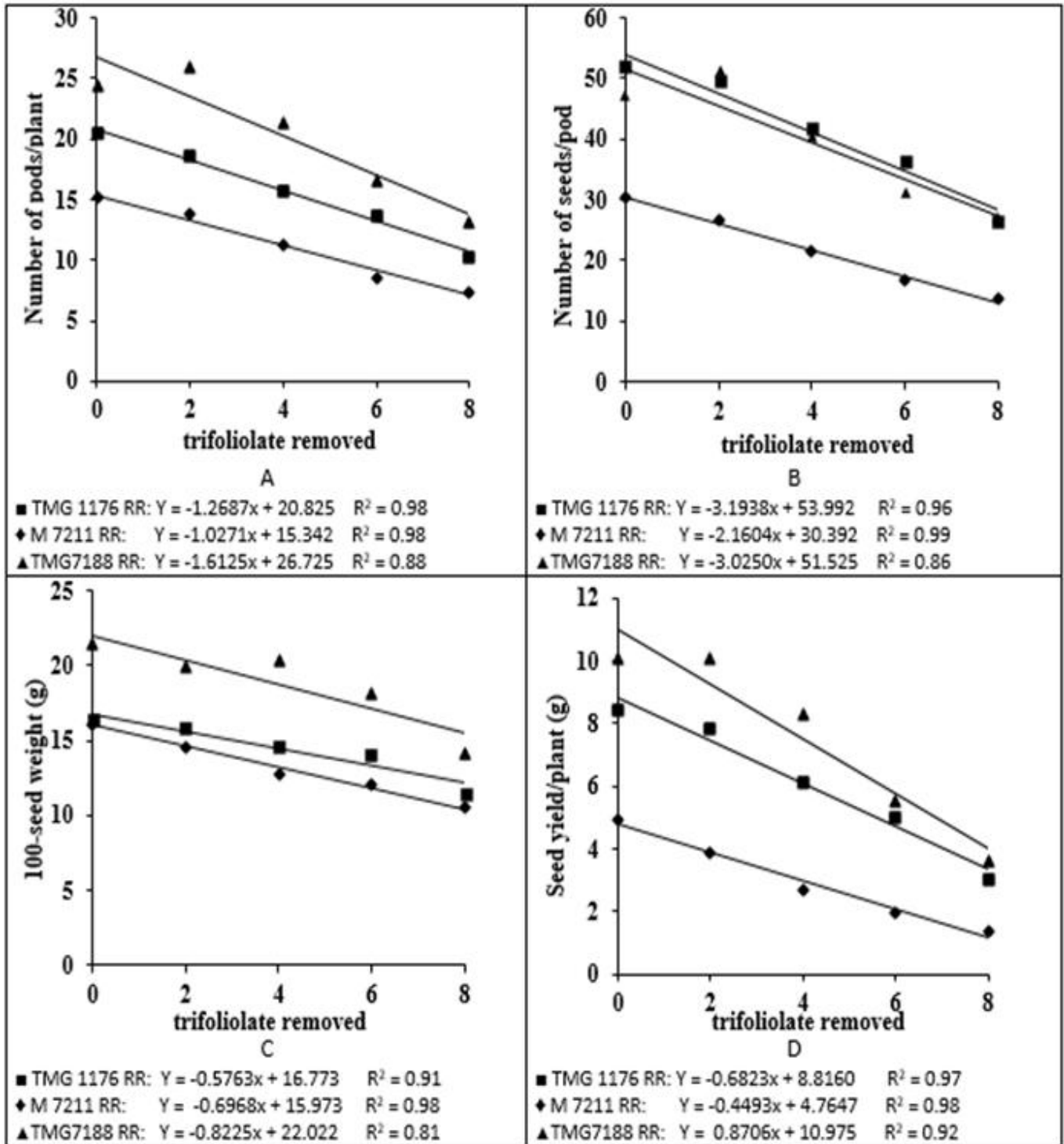
Figure 1. Number of pods/plant (A), number of seeds/plant (B), 100-seed weight (C) and seed yield / plant (D) in three soybean cultivars subjected to removal of trifoliolate leaves from the bottom to the top.

the abortion of pods resulting in lower total number of seeds/plant. In Figure 2 B it can be observed a linear decrease in the number of seeds/plant at R3 and R5 stages.

In the comparison of stages within each quantity of removed trefoil (Table 4) it was noted that there was a decrease from the loss of four trefoils, wherein in R3 stage, to decrease in yield was significantly greater

Table 3. Number of pods/plant, number of seeds/pod, 100-seed weight and seed yield/plant (g plant[-1]) in soybean cultivars subjected to removal of trifoliate leaves from the bottom to the top[1].

Parameter	Defoliation level (number of removed trifoliolate leaves)				
	0	**2**	**4**	**6**	**8**
Cultivar	Number of pods per plant (LSD[2] = 2.82)				
TMG 1176 RR	20.45 [B]	18.70 [B]	15.62 [B]	13.66 [B]	10.29 [B]
M 7211 RR	15.08 [C]	13.83 [C]	11.20 [C]	8.62 [C]	7.41 [C]
TMG 7188 RR	24.50 [A]	25.95 [A]	21.33 [A]	16.45 [A]	13.12 [A]
	Number of seeds per plant (LSD = 5.51)				
TMG 1176 RR	51.79 [A]	49.62 [A]	41.87 [A]	36.25 [A]	23.54 [A]
M 7211 RR	30.29 [B]	26.66 [B]	21.37 [B]	16.79 [B]	13.62 [B]
TMG 7188 RR	47.20 [A]	51.20 [A]	40.58 [A]	31.12 [A]	27.00 [A]
	100-seed weight (g) (LSD = 1.52)				
TMG 1176 RR	16.36 [B]	15.84 [B]	14.58 [B]	14.06 [B]	11.48 [B]
M 7211 RR	16.13 [B]	14.57 [B]	12.77 [C]	11.06 [C]	10.47 [B]
TMG 7188 RR	21.34 [A]	19.91 [A]	20.31 [A]	18.04 [A]	18.04 [A]
	Seed yield per plant (g plant[-1]) (LSD = 0.962)				
TMG 1176 RR	8.43 [B]	7.86 [B]	6.07 [B]	5.03 [A]	3.02 [A]
M 7211 RR	4.90 [C]	3.89 [C]	2.69 [C]	1.95 [B]	1.38 [B]
TMG 7188 RR	10.04 [A]	10.06 [A]	8.24 [A]	5.47 [A]	3.63 [A]

[1]Means followed by the same uppercase letter in the column do not differ at 5% probability by Tukey test. [2]LSD = Least Significant Difference.

Table 4. Number of pods/plant, number of seeds/plant and seed yield/plant (g plant[-1]) in soybean cultivars subjected to removal of trifoliate leaves from the bottom to the top at three reproductive stages[1]

Parameter	Defoliation level (number of removed trifoliolate leaves)				
	0	**2**	**4**	**6**	**8**
Stages	Number of pods per plant (LSD[2] = 2.82)				
R3	20.08 [A]	17.91 [B]	13.62 [B]	8.75 [B]	5.58 [B]
R5	20.29 [A]	19.41 [AB]	15.33 [B]	10.75 [B]	6.79 [B]
R6	19.66 [A]	21.16 [A]	19.20 [A]	19.25 [A]	18.45 [A]
	Number of seeds per plant (LSD = 5.51)				
R3	43.66 [A]	39.25 [B]	29.08 [B]	18.75 [B]	11.70 [B]
R5	44.37 [A]	41.66 [AB]	33.91 [B]	22.50 [B]	13.95 [B]
R6	41.25 [A]	46.58 [A]	40.83 [A]	42.91 [A]	41.50 [A]
	Seed yield per plant (LSD = 0.962)				
R3	8.04 [A]	6.98 [A]	4.97 [B]	3.09 [B]	1.64 [B]
R5	7.82 [A]	7.31 [A]	5.72 [AB]	3.44 [B]	1.74 [B]
R6	7.51 [A]	7.53 [A]	6.32 [A]	5.93 [A]	4.61 [A]

[1]Means followed by the same uppercase letter in the column do not differ at 5% probability by Tukey test. [2]LSD = Least Significant Difference.

than R6. However, with the loss of 6 and 8 trefoils, in both R3 and R5 stages had significant decrease in yield. In Figure 2C, it was observed that for defoliation in R3 and R5 stages there was a marked linear decrease in yield, reaching about 80% decrease with the removal of 8 trifoliate leaves. This is due to decrease in the number of pods, seeds and seed weight. Already a linear decrease in R6 stage is only resulting in the decrease of the seed weight. In this sense, should seek to control until R6 stage, because for many producers, at that stage the

A

■R3: Y = -1.9084x + 20.825 R^2 = 0.98
♦R5: Y = -1.7833x + 21.650 R^2 = 0.98
▲R6: Not signicant

B

■ R3: Y = -4.220x + 45.375 R^2 = 0.98
♦ R5: Y = -4x + 47. 283 R^2 = 0.96
▲R6: Not signicant

C

■ R3: Y = -0.8342x + 8.2832 R^2 = 0.99
♦ R5: Y = -0.8022x + 8.4178 R^2 = 0.96
▲R6: Y = -0.3659x + 7.8557 R^2 = 0.92
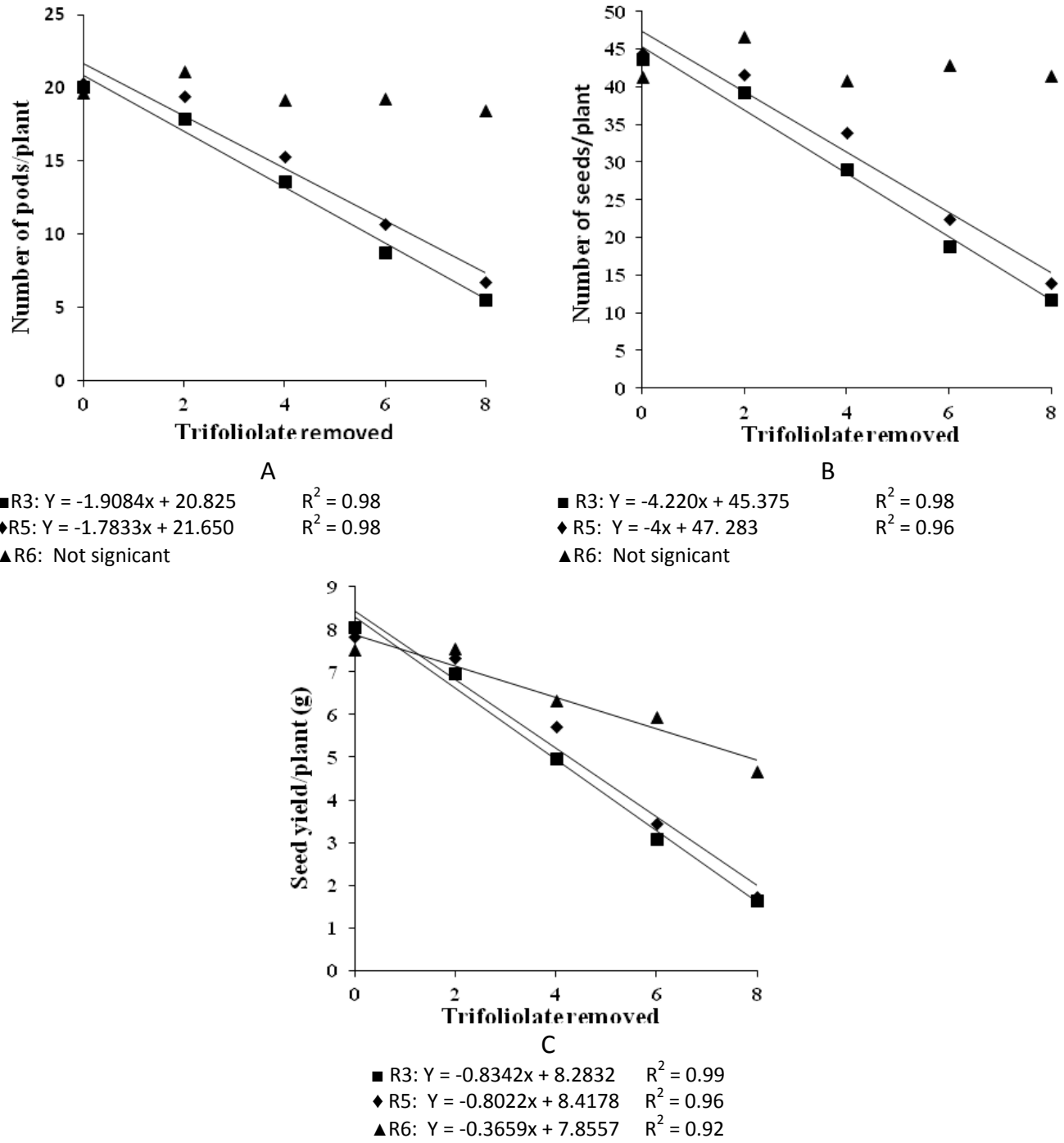
Figure 2. Number of pods/plant (A), number of seeds/plant (B) and seed yield/plant (D) in three soybean cultivars subjected to removal of trifoliolates leaves from the bottom to the top.

severity of soybean rust would not result in more lost in yield. However, depending on the crop situation, the control should be still performed. Gasparetto et al. (2011) observed that the fungicides application promoted gains in seed yield (kg ha^{-1}), which ranged from 30 to 59% compared to control, generating revenue in excess of 77 to 210%.

Comparing the control regarding the removal of 8 trifoliate leaves in R6 stage, the decrease was about 38%, an unacceptable loss to a crop that is already ending the cycle and has received all necessary management. To the 100-seed weight, there was effect only ofn the defoliation stage (Table 5). In the R3 stage the 100-seed weight was significantly higher than in the

Table 5. 100-seed weight of soybean cultivars in three defoliation stages[1].

Defoliation stage[1]	100-seed weight (g)
R3	16.12 [A]
R5	15.46 [AB]
R6	14.79 [C]
LSD[2]	0.682

[1]Means followed by the same uppercase letter in the column do not differ at 5% probability by Tukey test. [2]LSD = Least Significant Difference.

R6 stage.

DISCUSSION

The soybean rust, in severe cases, when the disease reaches the soybean plant in pod formation stage or early seed filling can cause abortion and the drop pods, resulting in up to total yield loss (EMBRAPA, 2011). According to Egli (2010), the soybean plant has two mechanisms to adjust the number of pods and seeds with the same availability of assimilates: i) The flower production varies with changes in environmental conditions and among cultivars; and ii) not all flowers produce pods and not all pods reach up to maturation. However, the abortion of pods is enhanced when plants undergo major environmental changes (like a stress period caused by defoliation) on flowering and seed filling.

The similarity between defoliation in the two different stages (R3 and R5) is because in the R3 stage the plant starts pod formation, and the stress by defoliation increases the abortion of these pods, however, the plant still has a specified period for forming and filling of some pods. In R5 stage, the pods are all already formed; however, because they are still at the beginning of photosynthate translocation to the seeds, increasing the defoliation also causes abortion, since the sources are removable. Thus, the plant just keeps the amount of pods that it will be able to fill the seeds with assimilates from the leaves (sources) that remain, unlike the defoliation caused by more advanced stage, where the seeds are reportedly in the process of filling and this negative effect would be in the weight of these seeds.

The soybean, as well as other species, has a strategy to leave progeny, in this case the stress caused by defoliation at R3, causes rearrangement in yield components, in order to maintain quality seed. Thus, the plant aborts most of their pods and retains only those which have the ability to translocate photoassimilates from the remaining leaves, which results in keeping the seed size. Especially in this case, where two of the three cultivars are determinate growth type, wherein R3

stage the total of leaves have been issued. In R5 stage it happens pretty much the same; however, as the pods are already in early seed filling, the abortion of pods can be fewer. In the R6 stage seeds are all formed, filling the entire pod cavity, however, it had not yet fully completed receipt of photoassimilates from the leaves, which results in the largest decrease in the seed weight. This proves by the recommendation of desiccation in soybean that is in R7, precisely for no loss in seed weight and consequently in yield (EMBRAPA, 2010).

It is known that the pods reaching its maximum length and seeds that underwent by cell division rarely abort. However, after this phase, environmental changes may result in improper pod filling, requiring a change in the size of the seed corresponding to the availability of assimilates (Egli, 2010), that is, the seeds cannot grow without assimilates availability and stress caused by loss of leaves in R5 and R6 stages interfering directly in seed filling.

There are two sources of assimilates for seed filling: current photosynthesis and remobilization of carbohydrate reserves. The contribution of carbohydrate reserves is apparently very low (<15% of the total seed weight) so, the maintenance of the leaves is essential to maintain the photosynthetic rate (current photosynthesis) during this phase (Egly, 2010).

The leaves removal interferes directly in leaf area index (LAI) of the plant. The soybean plant normally reaches a value higher than the critical LAI (95% absorption of sunlight). Thus, the plant may lose part of its leaves without seriously affecting its performance (Weber and Cadwell, 1966). However, it should be considered that a substantial loss always smacks a negative effect, especially if it occurs at a stage of advanced development, that is, during the reproductive phase, when it is no longer possible to replace the leaf area lost by the new vegetative growth. In this case, varieties with a tendency to have more branches, offer certain advantage (Miyasaka and Medina, 1981).

According to Carretero (2011), much of the variation in the cultivars cycle is associated with differences in the vegetative phase, which is not directly connected to yield. Increase in the leaf area does not increase canopy

photosynthesis or the growth rate of the crop, after the radiation interception reaches a maximum, there is no benefit in production with higher rates of leaf area.

One explanation for a very marked loss is linked to the growth type of cultivar. It is known that the R6 stage is defined when at least one pod in the last four nodes is completely filled, but still green. However, it is also known that in the cultivars of determinate growth type, the maturation begin from the top to bottom, that is, when pods in the last four are already at R6, most pods in the middle and bottom part of the plant are still in a later filling stage, which causes further loss. In contrast, in cultivars with indeterminate growth type, which maturation occurs from the bottom up, in this case, when there are pods at R6 stage in the last 4 nodes, it is certain that the pods below are already in an advanced stage, that is, there will be no loss in the yield.

In this study, two of the three cultivars are determinate growth type, which may have influenced this linear decrease in R6. In practice, in crop visit, usually technicians and producers usually observe the pods in the top of plant, for decision making if it would or not, do one more application to disease control, which may be affecting the final seed weight and consequently seed yield.

After the pathogens infect the bottom leaves, there is acceleration of falling leaves. Oliveira and Antuniassi (2011) under curative control conditions, with evaluation one day before the application, noted that the average severity in the lower third was of 35.9% (between 28.9 and 42.8%) while the upper third was 4.57% (varying between 2 and 7%), whereas the 90% CI. Andrade and Andrade (2002) obtained results which showed that in the chemical control of soybean rust, just a delay of seven days in the fungicide application (after detection of the disease), was sufficient for the increase in defoliation at 82%. With a delay of 14 days, defoliation increased by 155%.

Conclusions

As defoliation intensity rises from the bottom to the top during the reproductive stages (R3, R5, R6) studied, there is a linear decrease in plant yield, reaching on the highest defoliation level, loss of 79.6, 77.7 and 38.6% in R3, R5 and R6, respectively. The damage simulation by soybean rust through defoliation showed the severity in the reduction in the leaf area and its consequent reflection in the yield.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Effect of foliar application with salicylic acid on two Iranian melons (*Cucumis melo* L.) under water deficit

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Scarcity of water is a severe environmental constraint to crop production in arid and semi arid region. Salicylic acid (SA) plays an important role in the regulation of plant growth in response to environmental stresses. In this study, the effect of SA (0, 70 and 100 ppm) was investigated on growth and yield of two Iranian melons (Khatooni and Ghasri cultivars) under drought stress (W100%, W80% and W60%). Experimental design was a split factorial based on complete block design with four replications. Results showed average fruit weight, yield and fruit ripening duration were decreased by increasing water restriction but total soluble solid (TSS) and proline content increased. Salicylic acid increased chlorophyll content (SPAD), fruit ripening duration and TSS than control. Different water treatments and salicylic acid levels had no significant effects on number of fruit per plant.

Key words: Drought stress, melon, salicylic acid, total soluble solid (TSS), yield.

INTRODUCTION

Drought stress cause adverse effects on plant growth and productivity of crops. Drought stress cause an increase of solute concentration in environment, leading to an osmotic flow of water out of plant cells (Taheri-Asghari et al., 2009). Plants respond to environmental stress by induction of various morphological, biochemical and physiological responses. Sayyari et al. (2013) reported that drought stress decreased fresh weight, dry weight, leaf area and relative water content (RWC) but increased proline content in lettuce. Salicylic acid, a ubiquitous plant phenolic compound, has been reported to regulate a number of processes in plants (Hayat et al., 2008). It can improve plant growth under drought conditions and other stresses (Senaratna et al., 2000).

The salicylic acid (SA) increased the leaf area and dry matter production in corn and soybeen (Khan et al., 2003) and *Brassica junca* (Fariduddin et al., 2003). Exogenous application of SA improved the drought tolerance of wheat (Horvath et al., 2007). Jamali et al. (2011) reported that SA increased root and shoot fresh weight and yield of strawberry. Ghaderi et al. (2015) reported that SA increased leaf area, leaf number, proline content and yield in strawberry under drought stress. In another study on tomato under drought condition, SA increased proline content, RWC, SPAD and decreased electrolyte leakage (Hayat et al., 2008). This experiment was conducted to asses if SA could ameliorate the adverse effect of water deficit on melons.

Table 1. Mean squares from the analysis of traits.

S.V	DF	L.A	SPAD	P	NFPP	F.W	F.R	TSS	Y
R	3	0.02^{ns}	1.14^{ns}	0.0006^{ns}	0.20^{ns}	0.19^{ns}	2.78^{ns}	0.21^{ns}	20.22^*
W	2	8.80^{**}	76.98^{**}	0.5857^{**}	1.43^{ns}	11.14^{**}	292.68^{**}	4.88^*	704.43^{**}
E_w	6	0.02	1.39	0.0011	0.56	0.56	0.51	0.88	4.15
V	1	0.20^{ns}	15.13^{**}	0.0328^{**}	0.35^{ns}	0.03^{ns}	280.06^{**}	17.50^{**}	10.63^{ns}
SA	2	2.00^{**}	46.93^{**}	0.0321^{**}	1.06^{ns}	0.55^{ns}	261.43^{**}	5.72^{**}	2.14^{ns}
W V	2	0.06^{ns}	0.55^{ns}	0.0075^*	0.43^{ns}	0.01^{ns}	0.35^{ns}	0.95^{ns}	6.03^{ns}
W SA	4	0.10^{ns}	098^{ns}	0.0034^{ns}	0.24^{ns}	0.10^{ns}	6.78^{**}	0.51^{ns}	2.76^{ns}
V SA	2	0.06^{ns}	0.96^{ns}	0.0011^{ns}	0.06^{ns}	0.10^{ns}	8.43^{**}	0.21^{ns}	5.07^{ns}
W V SA	4	0.03^{ns}	0.49^{ns}	0.0002^{ns}	0.20^{ns}	0.08^{ns}	3.66^*	0.15^{ns}	2.67^{ns}
E_{WvSA}	45	0.07	0.82	0.0018	0.50	0.42	1.25	0.36	3.06

** and * significant at 1% and 5% level respectively and ns no significant.

MATERIALS AND METHODS

In order to evaluate the effects of SA under drought stress on two Iranian melons, a split factorial experiment based on complete block design with four replications was conducted in Torbat-e-jam (Longitude: 60˚48′, latitude: 35˚ 31′, altitude: 928 meters, with semi-arid climate, hot summers and cold winters) on sandy-loam soil field. Water treatment (W) in 3 levels (100, 80 and 60% water requirement) was considered as a main plot. SA (0, 70 and 100 ppm) with Khatooni (V1) and Ghasri (V2) cultivars were considered as a sub plot in a factorial design. Seeds of melon were planted in 1 may 2014. Six meters distance existed between every main plot and 3.5 m was between sub plots. Plants irrigated every 7 days. The first SA spraying was carried out when fruits had 10 cm length and after 20 days the second spraying was done. After one week leaf samples were collected for measuring traits.

Water requirement calculated base on a thirty years period weather of Torabt-e-jam, soil and water analysis and date of planning by OPTIWAT software was planned by Alizadeh professor of irrigation (Alizadeh and Kamali, 2009). Water requirement was calculated 9640 m^3/ha. In addition daily water requirement was calculated and the amount required for 7 days was put together and the water needed was measured by a volume counter. In this experiment leaf area (L.A), SPAD, proline content (P), number of fruit per plant (NFPP), average fruit weight (F.W), fruit ripening (F.R), total soluble solid (TSS) and yield (Y) were measured. The chlorophyll in the fresh leaf samples was measured by using Minolta chlorophyll meter (SPAD-502-Minolta, Japan). Proline content was estimated by the method of Bates et al. (1973). The plant material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 10,000 rpm. The supernatant was used for the estimation of the proline content. The reaction mixture consisted of 2 ml of acid ninhydrin and 2 ml of glacial acetic acid, which was boiled at100°C for 1 h. After termination of reaction in ice bath, the reaction mixture was extracted with 6 ml of toluene, and absorbance was read at 520 nm. Fruits harvested after ripening and weighed for every plot. Analysis of variance was carried out using Minitab software and Duncan's multiple range test calculated at 5% level of probability.

RESULTS AND DISCUSSION

Analysis of variance showed (ANOVA) that water treatments had significant effects on all traits unless NFPP. SA and cultivar had significant effects on SPAD, F.R, TSS and proline content at 1% level probability (Table 1). Results showed leaf area was decreased by increasing water restriction. Maximum (4.58 m) and minimum (3.43 m) leaf area were recorded by $W_{100\%}$ and $W_{60\%}$ respectively (Table 2). SA increased leaf area than control and the maximum leaf area (4.18) was obtained by SA_{100} (Table 2). There was no significant difference between two cultivars but Ghasri cultivar had more leaf area than Khatooni cultivar (Table 2). Interaction effects between water treatments with cultivars and with SA were not significant (Table 1) anyway the maximum leaf area was recorded by W_{100} with V_2 combination (4.58 m) (Table 3) and W_{100} with SA_{100} combination (4.96 m) (Table 4). Leaf area reduced by increasing water restriction, our results as well as Ghaderi et al. (2015), Barzgar et al. (2011) and Hayat et al. (2008). Growth is one of the sensitive physiological processes to drought, because cell expansion only occurs when turgor pressure is greater than cell wall pressure (Shao et al., 2008). In drought conditions, plants close their stomata to prevent the transpiration water loss (Mansfield and Atkinson, 1990). They may result in response to decrease in leaf water potential (Ludlow and Muchow, 1990). Also it decreases the inflow of CO_2 into the leaves, so CO_2 assimilation decreased (Shao et al., 2008).

Mean square of simple effects (Table 2) showed that the highest chlorophyll content was seen in $W_{100\%}$ (47.21) and SA_{100} (46.73). There was significant difference between two cultivars, Ghasri cultivar had more SPAD (45.64) than Khatooni cultivar (44.73) (Table 2). SA increased SPAD in all treatments than controls (Table 4). Our results were in agreement with Singh and Usha (2003) and Elizabeth and Munne-Bosch (2008). SA probably prevents from action of chlorophyll oxidase enzymes therefore it will be impediment chlorophyll breakdown, for this respect increased photosynthesis.

Proline content increased by drought stress, the maximum proline content was obtained by $W_{60\%}$ (1.558 mgr/gr fw) also proline increased by increasing amount of SA (Table 2). There was significant difference between

Table 2. Means comparison of simple effects for traits.

Treatment	L.A (m^2)	SPAD	Proline (mgr/gr fw)	NFPP	F.W (Kg)	F.R (Days)	TSS (%)	Y (t/ha)
$W_{100\%}$	4.58[a]	47.21[a]	1.249[c]	4.00[a]	4.09[a]	93.54[a]	11.57[b]	29.533[a]
$W_{80\%}$	3.67[b]	44.55[b]	1.365[b]	4.08[a]	3.52[b]	89.54[b]	12.25[a]	25.347[b]
$W_{60\%}$	3.43[c]	43.80[b]	1.558[a]	3.63[a]	2.73[c]	86.58[c]	12.43[a]	18.785[c]
V_1	3.80[a]	44.73[b]	1.369[b]	3.97[a]	3.47[a]	87.92[b]	11.59[b]	24.939[a]
V_2	3.96[a]	45.64[a]	1.412[a]	3.83[a]	3.43[a]	91.86[a]	12.58[a]	24.171[a]
Control	3.61[c]	44.01[c]	1.360[b]	3.71[a]	3.33[a]	86.46[c]	11.71[b]	24.629[a]
SA_{70}	3.89[b]	44.81[b]	1.380[b]	3.88[a]	3.39[a]	90.17[b]	11.91[b]	24.226[a]
SA_{100}	4.18[a]	46.73[a]	1.431[a]	4.13[a]	3.62[a]	93.04[a]	12.64[a]	24.810[a]

Means in the same column with different letters differ significantly at 0.05 probablitiy level according to Duncan's multiple rang.

Table 3. Means comparison of interaction effects between water treatments and cultivars for traits.

Treatment		L.A (m^2)	SPAD	Proline (mgr/gr fw)	NFPP	F.W (Kg)	F.R (Days)	TSS (%)	Y (t/ha)
W_{100}	V_1	4.58[a]	46.65[b]	1.231[d]	3.92[ab]	4.11[a]	91.50[b]	10.90[e]	29.415[a]
	V_2	4.64[a]	47.77[a]	1.267[d]	4.08[ab]	4.06[ab]	95.58[a]	12.25[bc]	29.651[a]
W_{80}	V_1	3.60[b]	44.02[d]	1.359[c]	4.25[a]	3.53[b]	87.50[d]	11.73[d]	26.232[b]
	V_2	3.74[b]	45.08[c]	1.371[c]	3.92[ab]	3.50[b]	91.56[b]	12.77[a]	24.463[c]
W_{60}	V_1	3.33[c]	43.52[d]	1.518[b]	3.75[ab]	2.76[c]	84.75[e]	12.15[cd]	19.171[d]
	V_2	3.53[bc]	44.08[d]	1.599[a]	3.50[b]	2.71[c]	88.42[c]	12.71[ab]	18.399[d]

Means in the same column with different letters differ significantly at 0.05 probablitiy level according to Duncan's multiple rang.

two cultivars, Khatooni cultivar had less (1.369 mgr/gr fw) proline than Ghasri cultivar (1.412 mgr/gr fw) (Table 2). Interaction effects between water treatments and cultivars were significant. The maximum proline content (1.599 mgr/gr fw) recorded by $W_{60\%}$ with V_2 combination (Table 3). Interaction effects between W and SA were not significant anyway in all treatments SA increased proline accumulation (Table 4). Because SA acts as a signalling molecules to active the signalling cascades by ABA, H_2O_2 and Ca^{2+}. Theses cascades then active the synthesis of specific protein kinases and active more responses such as changes in gene expression. Also changes in plant metabolism including synthesis and accumulation antioxidants and osmoprotectants such as proline (Farooq et al., 2009).

Analysis of variance (Table 1) showed all of the treatments were not significant on NFPP but only water treatments had significant effects on fruit weight. The maximum (4.09 Kg) and minimum (2.73 Kg) fruit weight were obtained by $W_{100\%}$ and $W_{60\%}$ respectively. In this study distinguished SA had no significant effect on fruit weight anyway SA increased fruit weight (Table 2).

Ghaderi et al. (2015) reported that SA increased fruit weight in strawberry.

Fruit ripening was decreased by increasing water stress. The maximum (93.54 days) and minimum (86.58 days) fruit ripening period was recorded by $W_{100\%}$ and $W_{60\%}$ respectively (Table 2). Between cultivars was significant difference, Ghasri cultivar late ripening (91.86 days) than Khatooni Cultivar (87.92 days) (Table 2). Fruits were treatments with SA more lately ripening than control and there were significant difference between treatments (Table 2). Interaction effects between water treatments and SA were significant. The maximum period (95.88 days) of fruit ripening was recorded by $W_{100\%}$ with SA_{100} combination and in all combinations fruit ripening increased as the concentration of SA increased (Table 4). The same results were reported by Lolaei et al. (2012). Probably SA inhibited ethylene production in fruits. Many researchers proposed the role of SA as an antagonist to ethylene action (Marissen et al., 1986; Leslie and Romani, 1988; Shafiee et al., 2010). Analysis of variance (Table 1) showed that water treatments had significant effects on TSS (P≤0.05). $W_{60\%}$ had more TSS (12.43%)

Table 4. Means comparison of interaction effects between water treatments and SA for traits.

Treatment		L.A (m^2)	SPAD	Proline (mgr/gr fw)	NFPP	F.W (Kg)	F.R (Days)	TSS (%)	Y (t/ha)
W$_{100}$	Control	4.15^c	45.70^c	1.222^e	3.88^{ab}	4.05^{ab}	91.13^c	11.03^e	30.31^a
	SA$_{70}$	4.62^b	46.79^b	1.256^e	4.00^{ab}	3.88^{ab}	93.63^b	11.38^{de}	28.92^a
	SA$_{100}$	4.92^a	49.14^a	1.268^e	4.13^a	4.33^a	95.88^a	12.31^{abc}	29.36^a
W$_{80}$	Control	3.48^d	43.73^d	1.330^d	4.00^{ab}	3.38^{bcd}	86.00^d	11.81^{cd}	24.79^b
	SA$_{70}$	3.60^d	44.11^d	1.359^d	3.88^{ab}	3.53^{bc}	90.00^c	12.00^{bcd}	25.29^b
	SA$_{100}$	3.93^c	45.81^c	1.406^c	4.38^a	3.66^{ab}	92.63^b	12.94^a	25.96^b
W$_{60}$	Control	3.19^e	42.61^e	1.529^b	3.25^b	2.56^e	82.25^e	12.28^{abc}	18.79^c
	SA$_{70}$	3.44^{de}	43.54^d	1.526^b	3.75^{ab}	2.76^{de}	86.88^d	12.34^{abc}	18.47^c
	SA$_{100}$	3.66^d	45.25^c	1.619^a	3.88^{ab}	2.87^{cde}	90.63^c	12.66^{ab}	19.10^c

Means in the same column with different letters differ significantly at 0.05 probablitiy level according to Duncan 's multiple rang.

and W$_{100\%}$ (Table 2). According to the results V$_2$ had more (12.58%) TSS than V$_1$ (11.59). The SA$_{100}$ significantly increased (12.64%) TSS than SA$_{70}$ (11.91%) and control (11.71%) and wasn't seen significant difference between SA$_{70}$ and control (Table 2). Our results were in agreement with Karlidag et al. (2009) and Javaheri et al. (2012).

Between treatments only water treatment had significant effect on yield. The yield decreased by water restriction. The highest yield was obtained by W$_{100\%}$ (29.533 t/ha) (Table 2). Our results were in agreement with Barzgar et al. (2011); Ghaderi et al. (2015); Hayat et al. (2008); Mirabad et al. (2013). There was no significant difference between SA treatments anyway SA$_{100}$ had more yield (24.810 t/ha) than SA$_{70}$ and control (Table 2). According to the analysis of variance (Table 1), there was no significant difference between two cultivars. Interaction effects between water treatments and cultivar showed that in W$_{100\%}$ treatments V$_2$ had more yield than V$_1$ but by increasing water stress V$_1$ had more yield than V$_2$ (Table 3). Results showed by increasing drought stress proline content increased especially in V$_2$ (Table 3). It is concluded that the tolerance of V$_1$ is more than V$_2$ to drought stress genetically.

Conclusion

Iran has high genetic variation of melon. More study need to identify tolerance genotype to breeding programs. SA increased level of antioxidant system both under drought stress and without stress conditions. We are suggesting evaluating different levels of SA to increase yield and quality for melon.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Determination of grazing time with relationships between grass layer height and biomass change in natural pastures

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This research work was conducted in Karahisar village of Tekirdağ city during the years of 2010 and 2011. The basic purpose of this study was to determine the starting dates of grazing in pastures, and the relationships between grass layer height and biomass change in protected, as well as, the grazed areas of natural pastures of the said village. In pastures, 16 different points had been marked in 4 rangelands on tetrad sampling areas for measurements. The measurements were carried out with 10-day intervals between the dates of 10th March to 20th July. Height range of the grass layer in pastures had been changed from 5.04 to 48.73 cm in grazed pasture, while 6.47 to 56.83 cm in case of protected pasture. Grass layer height was reached to the highest range between 10 and 20 June in grazed pasture, but in 20th June in protected pasture. A significant relationship had been found between grass layer height and sampling dates of grazed pasture ($R^2 = 0.9773$) and protected pasture ($R^2 = 0.9808$). The highest biomass change was observed in grazed pasture (242.39 kg/ha) in 10th of June, while in protected pasture (275.59 kg/ha) in 20th of June. The results of this evaluation have been reached to a conclusion that the appropriate grazing would be started between 20 and 30th of April when vegetation sorted to 15 cm in pastures.

Key words: Pasture, grass layer height, fodder yield, biomass change.

INTRODUCTION

Meadow and pasture ecosystems have different potential of appearance and production from each other in different ecological conditions of the earth using solar energy with its own internal mechanisms (Altın et al., 2011a). Pastures play an indispensable role in the continuation of the food chain by meeting the needs of heterotrophic organisms, while performing this function.

Pastures occupy 25% of the world's land and 18.8% of the territory of Turkey (Anonymous, 2012a). Turkey's total livestock assets are equivalent to 14.3 million animal

units (AU) (Anonymous, 2013). For healthy and economical livestock breeding, the animals need approximately 65.3 million tons of quality roughage/forage. 26.8 million tons of this forage are met from forage crops (Ak, 2013), and also 11.2 million tons from meadows and pastures. After all, Turkey's need for roughage is up to 27.3 million tons (up to 27.3 million tons in Turkey are needed for quality roughage). Best way to cover the gap is to increase the production of pasture by using pasture improving techniques. For a successful pasture improvement, it is required to know the existing vegetation characteristics of pastures.

Pastures comprise several species of different ecological requirements. The development of species in different periods of growing season provides continuity of vegetation forage quality. This is defined as periodicity and it reduces competition among plant species in pastures. However, the grassland vegetation types are in competition constantly in order to maintain growth or re-growth along with vitality (Çetiner et al., 2012). The competition in meadow and pastures occurs mostly in terms of water, nutrients and light. Competition is higher for these factors in the plant communities where dense population of taller plants exist. Species forming stolon, lumpy, rhizomes, shoots and the tangles compete for more areas (Erkovan et al., 2008). If plants show a need to any of these resources simultaneously, their growth, development and product-making capacity may be reduced. In addition, the competitiveness of plants varies depending on the grazing time and intensity (Alhamad and Alrababah, 2008; Çetiner et al., 2012). The high competition in plant communities of tall plants adversely affects the growth of some species (Şahin, 2007).

The vegetation of pastures in Turkey were classified as short (<60 cm), medium (60-120 cm) and tall (> 120 cm) plants by Davis (1965 -1985) (Davis, 1988). Bakır (1987) reported that the grazing maturity of pastures as 7.5 to 10, 15 and 20 cm for short, medium and tall plants; respectively. Terzioğlu and Yalvaç (2004) have found the grass layer height of the vegetation as 9.86 to 10.50 cm for short tall plants; for 13.24 to 15.63 cm the medium tall plants; 17.48 to 19.50 cm for the long tall plants in his recommended period for the beginning of the grazing pasture (10th of May). Difante et al. (2009) reported that when the average height of grass layer is 65 cm in Brazil, pre-grazing yield is 7.130 kg ha^{-1} and after grazing is 3.810 kg ha^{-1}.

The purpose of the study is to reveal the relationship between height of grass layer with biomass increase of the vegetation of protected and grazing pastures in spring green forage period (from 10 March to 20 July), and to determine the appropriate starting time of grazing. .

MATERIALS AND METHODS

This study was conducted in Karahisar village of Tekirdağ city

during the years of 2010 and 2011 on protected and grazing pastures.

Tekirdağ is located in the transition zones between Mediterranean, Central Anatolia continental and the Black Sea climate. The average temperature and total annual precipitation (2010 - 2011) is 15.4°C and 766.8 mm, respectively. Long-term (1965 - 2011) average temperature and total annual precipitation is 14.3°C and 589.8 mm, respectively (Figure 1). The average temperature and annual precipitation amounts of all months in the research years has been higher than the long term average (Anonymous, 2012b).

Vegetation of Thrace has characteristics of Anatolian steppe, Central Europe Mediterranean and Colchic vegetation (Dönmez, 1968). Studies in this region (Gür and Altın, 2011; Gür, 2008; Tuna, 2000) showed that dominant vegetation species were *Trifolium campastre, Medicago minima, Lotus corniculatus Dactylis glomerata, Lolium perenne, Chrysopogon gryllus, Koleria cristata, Vulpia ciliata, Eryngium campestre, Plantago lanceolata* and *Sanguisorba minor.* The rate of plant covered area was 79.06% in grazed pasture, 80.32% in the protected pasture, respectively. The grasses, legumes and the other families on botanical composition were determined as 48.69, 23.84 and 27.47% on grazed pasture, 50.30, 24.23 and 25.47% on protected pastures, respectively.

Soil samples were taken from the research areas and then the levels of pH, organic matter, P and K were measured. Organic matter ratio was 6.45, 2.66%, 8.50 ppm and 165.64 ppm on grazed pasture, while 7.72, 1.19%, 3.67 and 60.85 ppm on protected pasture, respectively. It was determined that grazed pasture was found slightly acidic and clayey. On the other hand, the protected pasture was slightly alkaline and loamy.

In this study, four sample parcels of 600 m^2 (30 m × 20 m) area each in grazing and protected pastures have been established. The measurements were taken from four rows in each of four designated sampling areas of the pastures. A total of 14 measurements were carried out using 10-days intervals for monitoring the grass layer height and biomass change between 10 March and 20 July. Grass layer height was measured from soil surface to the top end length of plant as "cm". To determine the biomass change, the aboveground of 50 × 50 cm = 0.25 m^2 area was harvested. Firstly, the fresh samples were dried in the shade, then dried into the oven at 70°C for 24 h until constant weight (Altın et al., 2007) and secondly, the weights were identified as kg /ha.

To assess the reliability of results, they were subjected to statistical analysis by SSPS 15.0 software package. Means were compared by using Duncan multiple comparison test.

RESULTS AND DISCUSSION

Grass layer height

The average height of pasture vegetation consisting of herbaceous species have demonstrated significant changes in the first growing season (March 10 to July 20), where measurements were made. Also, a significant differences (P <0.01) were found between the grass layer heights of grazing and protected pastures.

According to the average data of two years, grass layer height on grazing pasture at the first sampling (March 10) was measured as 5.04 cm. With the progression of the seasons, the plants continued to grow and increase in height was continued until June 20. The grass layer height of pasture on June 20 reached an average of
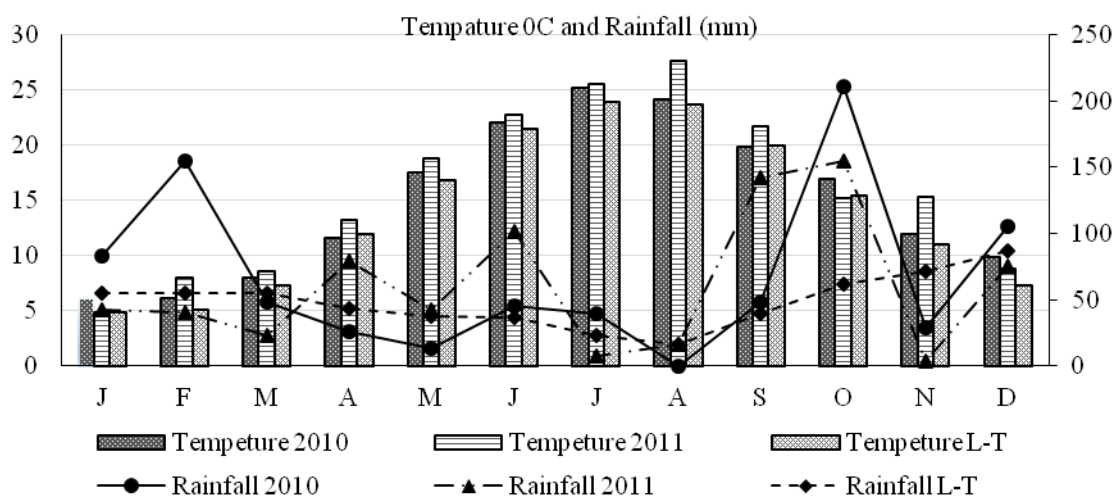
Figure 1. Data regarding to the average temperature (°C) and the total rainfall (mm) of Tekirdağ province

48.73 cm. Then, with increasing temperature and decreasing precipitation, plant growth were stopped, even depending on drying in the existing plant components, breakage and spillage was occurred. In the last measurement date (20 July), grass layer height was reduced to 41.14 cm (Figure 2). The pasture grass layer height of protected pasture in all sampling dates was more than that of grazing pasture. While, the average height of pasture vegetation was 6.47 cm on March 10, it reached to 56.83 cm on June 20 with the progress of growth. After this date, as well as grazing on pasture, grass layer height began to decrease, and a month later was reduced to 48.69 cm (Figure 3).

In pastures dominated by cool climate species, the maximum plant growth was observed in the spring season (Opitz von Boberfeld, 1994) hence, the grass layer touched the highest point of level in the said season of the same year (Bayraktar, 2012). However, growth of the cool climate plants start with rise of air temperatures above 0°C in the spring season (Serin and Tan, 2001) and this plants draws a curve S-shaped and slanted (Tosun, 1971). The initial slow growth are due to low air temperatures and insufficient photosynthetic area of the plants. Then, both increase in temperature of the air and soil, and preferring the products of photosynthesis instead of reserve nutrients by the plants with increasing in photosynthetic tissues provides acceleration in growth (Altın et al., 2011a). In the last period, with the gradual increase in air temperature and reduction in rainfall the effects of the summer drought is seen and the growth is under pressure. The plants growth rate varies according to type, time and environmental factors (especially temperature and soil moisture) (Koç, 2001) and cool climates forage grasses make their best growth at temperatures around 20°C (Miller, 1984). In contrast, in

general, the plants enter stress after 25°C, then their growth slows down and stops (Moser and Hoveland, 1996). So, the growth has slowed in the last period of developments coinciding with the beginning of summer season. Besides this unfavorable weather changes, an important part of cool climate plants begin to bloom towards the end of the spring.

In general, it was observed from this study that intense blooming range was between 10 and 30 May. With the beginning of flowering, vegetative growth was reduced, no new leaves (photosynthetic tissue) were sprouted and the movement of height increase stopped. After this period, the growth of grass layer height being decreased because of spending energy for flower, fruit and seed formation. The reduction in height of grass layer in the last period of this study was because of branches and leaves lose of the plants due to drying of plants entering drought stress and seed maturing. The height reduction after drying in plants causes more broken and spillage of generative branches. In this study, the first slow growth period in both pasture types lasted until April 30. From this date, growth has accelerated, this rapid growth has continued until June 10 on grazing pasture and until June 20 on protected pasture. The fastest growth on pastures has been in the month of May.

The drought of summer season in the region under normal circumstances begins from the June. During two years (2010, 2011) of our study, the June precipitation of both years (45.6 and 101.8 mm) was higher than that of long-term average rainfall (36.6 mm) (Figure 1). This situation has both prevented early onset of drought stress and helped to continue of plant growth. Even, the presence of water and rain amount in the pasture increases the annual production and extends grazing season (Erkovan et al., 2008).
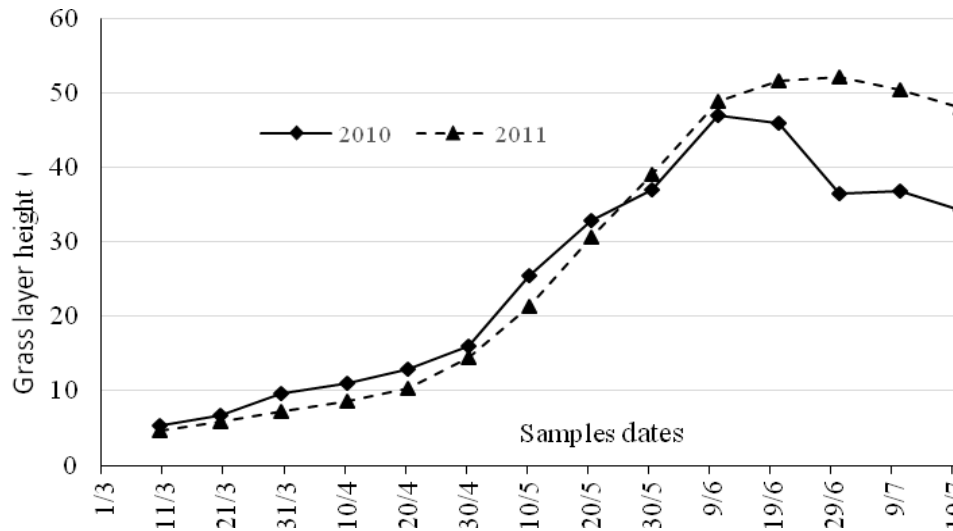
Figure 2. Variation occurred in grass layer height of grazing pasture in 2010 and 2011(P<0.01).
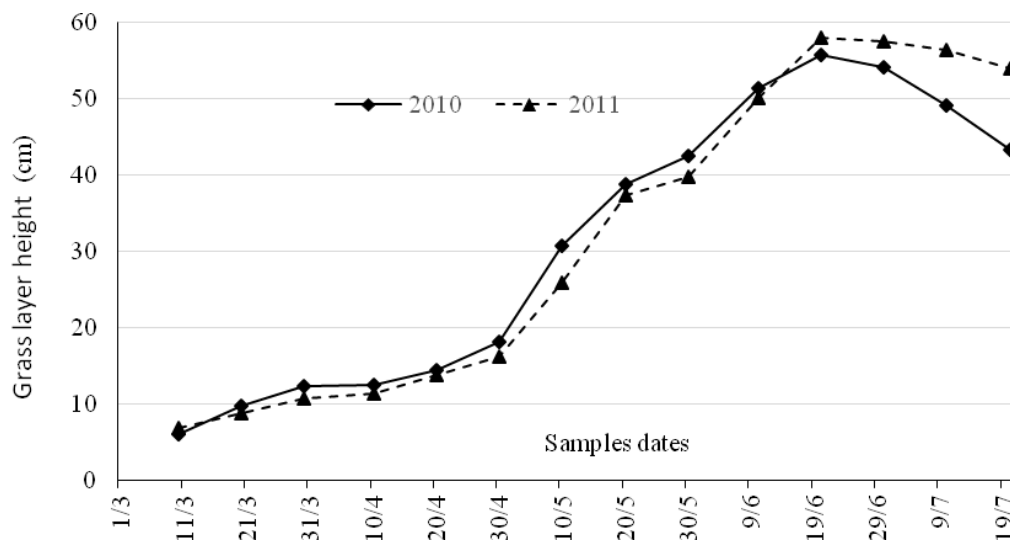
Figure 3. Variation occurred in grass layer height of protected pasture in 2010 and 2011(P<0.01).

Grass layer height, according to the years, showed a similar change. However, while the grass layer height of vegetation on grazing pasture in 2010 was significantly lower. The height difference of two years on protected pasture was not significant. As seen in Figures 2 and 3, grass layer height change is consistent with the two-year average.

Biomass change in the vegetation

Biomass, a total weight of green parts of the plant communities per unit area, is one of the most important features to determine the yield and capacity of grazing in pastures (Ünal and Aydoğdu, 2012). The weight of the history the highest above-ground biomass in pastures shows hay yield during growth (Bayraktar, 2012).

According to average data of two-years, biomass change of pasture vegetation was significant in the first growing season covering the spring and early summer seasons (Figure 4). Plant biomass in early March was determined by 852.9 kg ha^{-1} on grazing pasture. As in parallel with the growth and development of plants, the amount of organic biomass on pasture was continuously
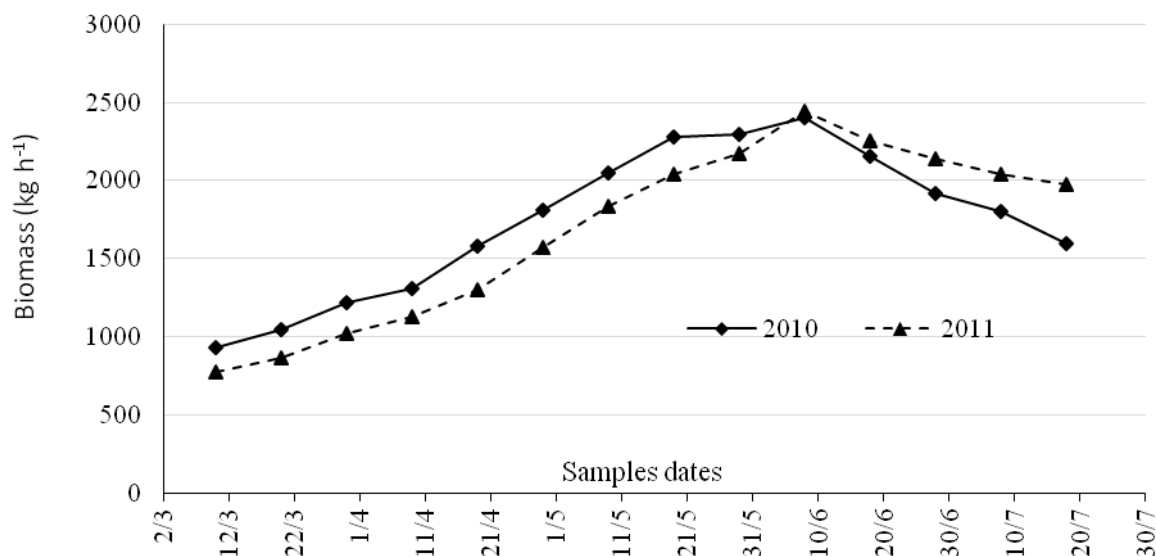
Figure 4. Variation occurred in plant biomass of grazing pasture in 2010 and 2011 (P<0.05).
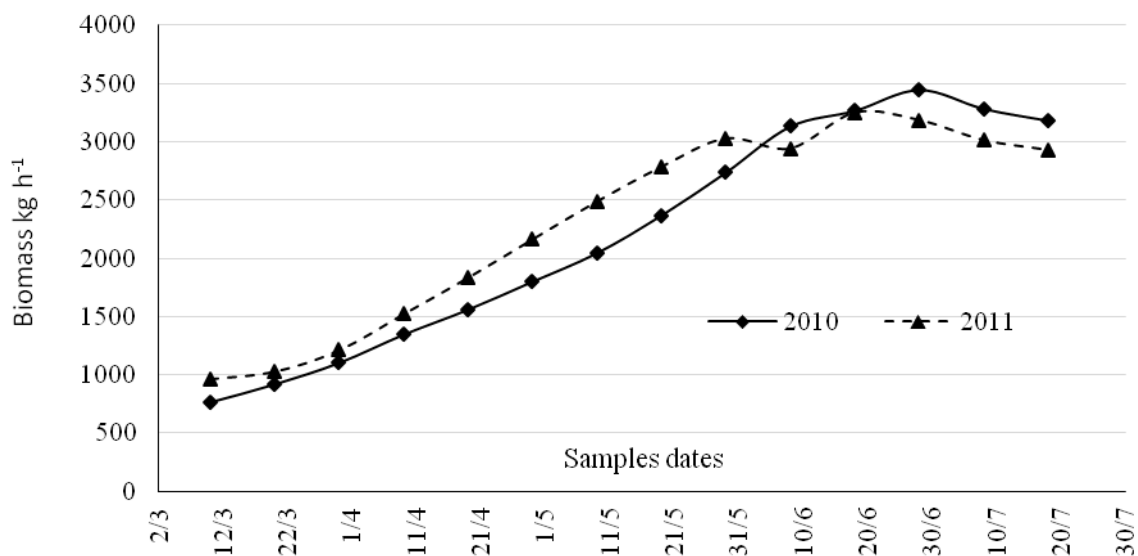
Figure 5. Variation occurred in plant biomass of protected pasture in 2010 and 2011 (P<0.05).

being increased and reached to 2423.9 kg ha^{-1} on June 10. Then, with continuous reduction of the plant biomass, it has fallen to 1786.0 kg ha^{-1} on July 20th. The biomass production in the spring accelerated after April 10. Similar changes were observed in the protected pasture. Here the plant biomass (859.8 kg ha^{-1}) was determined as close to the grazing pasture in the beginning of sampling. The biomass increase continued until June 20. However, the next increase after June 10 was not significant, because the biomass which was identified as 2744.6 kg

ha^{-1} on June 10 has been identified as 2755.9 kg ha^{-1} on June 20. From this date until 20 July, the plant biomass has continuously decreased. The biomass increase in the protected pasture has been a bit more after April 30 (Figure 5).

Since temperature and humidity are the most important factors in plants' organic material production, the maximum biomass increase in pastures was occurred in April and May when the temperature and soil moisture is optimum. In the protected pasture there was a significant

increase every 10-day measurement range from March 20 until June 10, because plants started over wintering with sufficient reserves of nutrients from previous growing season for many years. This situation occurred in the protected pasture. In this way, plants entering winter shows earlier and strong growth in the spring season. Since the effect of protection on plants in protected pasture, each sampling time in the growing season was observed to create more biomass. So, the highest plant biomass (yield of pasture) on grazing pasture 2423.9 kg ha^{-1}, has been identified with more than 14% in the protected pasture as 2755.9 kg ha^{-1}.

Because temperature and precipitation values affect both of vegetative growth and development, the effects of years on plant biomass were found significant (P <0.05) on both pastures. But, an adverse situation was occurred between two types of pasture. In fact, while average more plant biomass on grazing pasture was more in 2010, the plant biomass produced was higher in protected pasture in 2011 (Figures 3 and 4). Since the reasons of plant biomass change during growth period in pasture is similar to those of the change of grass layer, it is not discussed here separately. Furthermore, the data of this study is higher than hay yield in grazing and protected pastures in the same region in studies conducted by Altın and Tuna (1991) and Tuna (2000) which showed similarities with the detected values of Altın et al. (2007) and Gür and Altın (2011).

Relations between grass layer height and biomass

The relations between grass layer height and biomass of the pastures are discussed over a two-year averages. The relationship between grass layer height and biomass in both pasture types (R^2 = 0.9862 on grazing, R^2 = 0.9762 on protected) as found significant and positive (Figures 6 and 7). Depending on the horizontal and vertical development in plants, height and diameter width were increased and as a result, increase in biomass occurs. It has been demonstrated in a similar manner that there is a linear relationship between biomass and grass layer height (Anderson and Kothmann, 1982; Bayraktar, 2012; Gökbulak, 1997; Koç and Gökkuş, 1994; Nesheim, 1990) too. Cook and Stunbbendieck (1986) reported that the existence of a strong relationship between weight with sprout diameter and length according to regression analysis. As plant height increases, biomass is increasing. As vegetation reaches a height of 10 cm around, the biomass increase is accelerating, a height of 35 to 45 cm around the stands. Based on these bilateral relations, biomass of vegetation can be estimated according to height of pasture grass. Pasture yield can be estimated as 10-12 cm is 1200 to 1350 kg ha^{-1} year, 15-17 cm is 1600 to 1850 kg kg ha^{-1} year, 28-30 cm is 2100-2150 kg kg ha^{-1} year and exceeds of 50 cm is 2450 to

2750 kg kg ha^{-1} year. With these values, estimated rates of the covered regions of pastures with plants should be higher. The plant covered area ratios of pastures ranged from 79.1 to 82.5%. Sims and Singh (1978) reported that the annual aboveground net primary production of grazing of tall pastures is 28% more production than non-grazing pastures. Loeser et al. (2004) also reported that the grazing rangelands in semi-arid region provides 27 to 31% more production than in non-grazing pastures.

Grazing maturity

Grass layer height (Altın et al., 2011b) and the change in biomass of the plant provides information about grazing maturity. To reach to grazing maturity of plants, they must grow with products of photosynthesis instead of growing with reserving nutrients. Real growth of plants carries out with producing photosynthesis. Thus, the period of accelerated growth of plants in the spring is recognized as maturity grazing (Altın et al., 2011b; Ogden, 1980).

In this study, the height increase of the vegetation on grazing and protected pastures has accelerated on April 30 and on May 10, respectively (Figure 1). On the other hand, biomass increase on grazing and protected pastures is tended to increase more rapidly from April 20 (Figure 2). According to these results, it is appropriate to initiate the grazing of the pastures after April 20 in the pasture where the experiments were conducted. However, the dependence of plant growth to climatic factors and the fluctuations of weather conditions through years affect plant growth. Therefore, the researchers has expressed that the grass layer height would be more accurate handling in determining the maturity grazing. It is recommended to start grazing when average height of vegetation becomes 7.5 to 10.0 cm in pastures where short height species have been diffused (Altın et al., 2011b; Bakır, 1987).

In this study, grass layer height ranged from 15 to 17 cm according to the types of pasture in 30th of April. In contrast, the vegetation height has been reached to 10 cm on 10th of April. Here, because the physiological strengthening of plant is more important than the suggested height values in the literature, there are benefits to wait until the middle of April for grazing starting. Related to this subject, Koç and Gökkuş (1995) suggested that bolting together with symptoms of yellowing in the bottom leaves may be indicator for grazing maturity.

In decision-making for maturity grazing, the moisture content of the soil is also important in addition to the current state of the vegetation. In fact, during wet periods of pasture lands, the soils under grazing animal's hooves is compressed, consequently soil volume weight increases, infiltration rates are falling and young plants is broken off with roots (Mikhailova et al., 2000). Damage
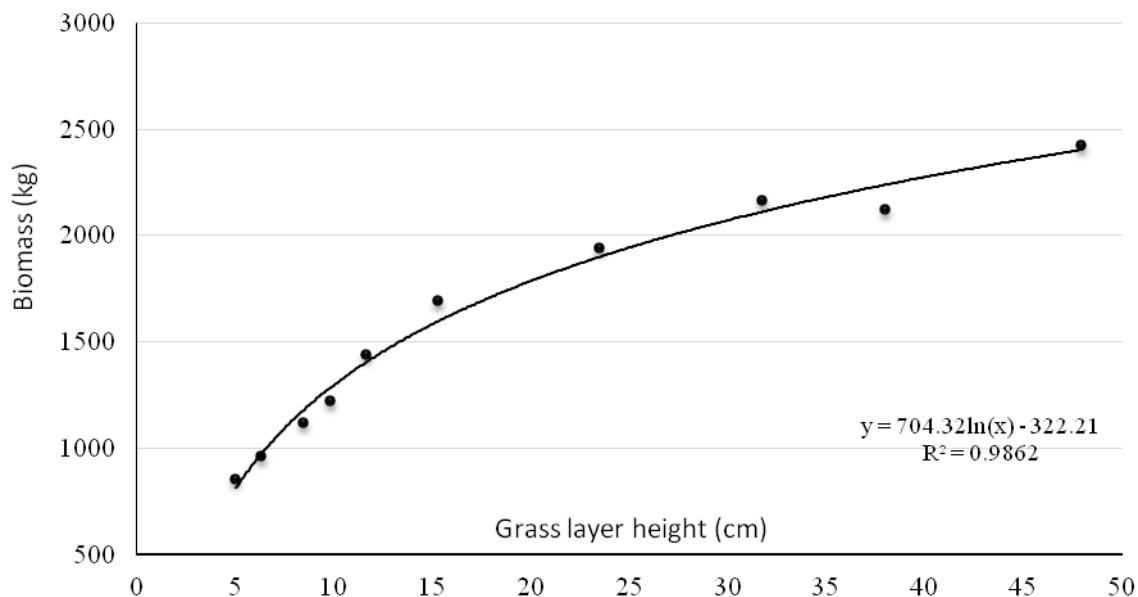
Figure 6. The relationship between grass layer height and plant biomass in grazing pasture.

$$y = 704.32\ln(x) - 322.21$$
$$R^2 = 0.9862$$
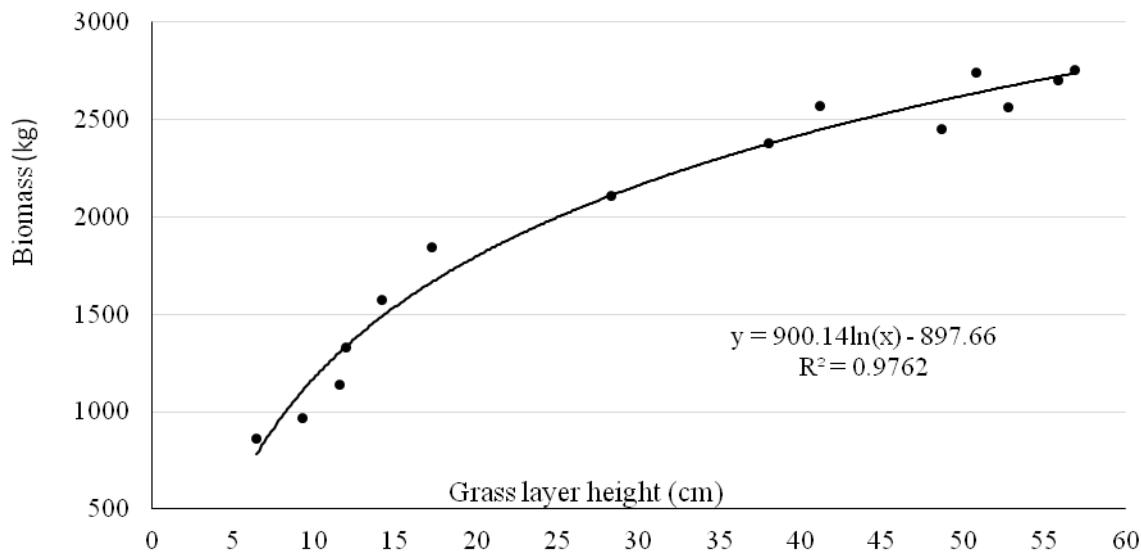
Figure 7. The relationship between grass layer height and plant biomass in protected pasture.

$$y = 900.14\ln(x) - 897.66$$
$$R^2 = 0.9762$$

caused due to chewing from grazing in early spring, water permeability of pasture land and plant covered area decreases and the species composition is changing (Bakoğlu and Koç, 2002). Thus, Babalık and Sönmez (2009) stated that initiation of grazing without endangering the continuation of the pasture plants life and without causing compaction of pasture soils, grasslands may also begin grazing when important pasture plants in spring could be grazing. It is recommended in other studies conducted in our country that beginning grazing in Erzurum is at the earliest on 20 May (Koç and Gökkuş, 1995) and in early May in Isparta (Babalık and Sönmez, 2009).

Conclusion

The following conclusions are being considered with the

help of a survey carried out for two years on grazing as well as protected pastures in Thrace region.

1. Dominant plants of pastures was short.
2. The grass layer height and biomass during plant growth was changed significantly. The high grass layer height and plant biomass (herb yield) in both pastures have been achieved between the dates of 10 to 20 June.
3. Appropriate time of grazing in pastures will be suggested between the dates of 20 to 30 April when the height of vegetation becomes about 15 cm.
4. Yield should be predicted according to the grass layer height. In pastures where plant cover occupies 80% of the region, 170 kg of forage is produced at the beginning of grazing season when an average of 15 cm of vegetation height has been obtained.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Effect of N fertilization on NPK content in castor bean under saline stress

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The high concentrations of salts in water, especially in semiarid regions, increase salt concentration in the soil and plant with the consequent decrease in NPK contents. Current study evaluates the influence of irrigation with water of different salinities, associated to nitrogen (N) fertilization, on NPK contents in castor bean cv. BRS Energia. An experiment was conducted for four months, in lysimeters under the field condition, in the municipality of Pombal, Paraíba - Brazil, using an Eutrophic Entisol of sandy loam texture ina randomized 5 × 4 factorial block design with three replications. Five levels of electrical conductivity of irrigation water (ECw=0.3, 1.2, 2.1, 3.0 and 3.9 dS m^{-1}) and four doses of N (70, 100, 130 and 160 mg kg^{-1}) were tested. The highest contents of potassium (K) in roots and leaves were obtained respectively with ECw of 0.3 and 2.2 dS m^{-1}. The N content in leaves was not influenced by factors under analysis. Increase in N dose decreased the plant contents of phosphorus (P) and K.

Key words: *Ricinus communis* L., electrical conductivity, regressions.

INTRODUCTION

The castor bean plant (*Ricinus communis* L.) of the family *Euphorbiaceae*is an oleaginous plant with high socioeconomic value, featuring products and by products used in the castor oil chemical industries and in agriculture, with a possible use as a biofuel. Oil from its seeds has approximately 90% ricinoleic fatty acid with special characteristics and energy contents. It is used in

industries as an ingredient in the manufacture of plastics, synthetic fibers, paints, enamels, lubricants and others (Almeida Júnior et al., 2009).

Castor bean seeds cv. BRS Energia were used as planting material. According to Silva et al. (2009), the cultivar is a vigorous genetic material, easily propagated, with a cycle of 130 days, low height (average 106 cm), with

Table 1. Physical and chemical characteristics of soil used in the assay

Chemical characteristics								
pH (H$_2$0) (1:2,5)	OM g kg^{-1}	P (mg kg^{-1})	K$^+$	Na$^+$	Ca^{2+}	Mg^{2+}	Al^{3+}	H$^+$
			(cmol$_c$kg^{-1})					
6.01	6.00	20.09	0.43	0.37	3.95	3.70	0.00	2.31

Physical characteristics

Granulometric fraction (g kg^{-1})			Texture class	Density (kg dm^{-3})	Total porosity	Water content (kPa)		AW dag kg^{-1}
Sand	Silt	Clay				33.42 dag kg^{-1}	1519.5	
860	100	40	SL	1.34	0.48	18.01	9.45	8.56

OM, organic matter: Walkley-Black humid digestion; Ca^{2+} and Mg^{2+}= calcium and magnesium ions extracted with KCl 1 mol L^{-1} at pH 7.0; Na$^+$ and K$^+$= sodium and potassium ions extracted with NH$_4$OAc 1 mol L^{-1} at pH 7.0; SL– sandy loam ; AW – available water.

semi-indehiscent fruits, oil content in seeds averaging 48% and an average productivity of 1.800 kg ha^{-1}.

The use of castor oil for the production of biodiesel is a hallmark in economic, environmental and social fields by increase in jobs and profits with the strengthening of agricultural economy, especially family agriculture, and the generation of opportunities due to an increase in agricultural areas in the semiarid northeastern regions in Brazil (Azevedo and Beltrão, 2007). However, precisely in this region the rainfallis very irregular and irrigation is the best method to guarantee agricultural production. Due to demands for a good water quality and the growing needs for the expansion of agricultural production worldwide, farmers have been compelled to use water with moderate to high salt contents in irrigation (Nobreet al., 2011).

High concentrations of salts in the soil may not only reduce the soil´s water potential but also may cause toxic effects in plants, such as functional disorders and damages in their metabolism (Debouba et al., 2006). The above-mentioned effects depend on many other factors such as plant species, cultivar, phenologic stage of crop, type of salts, intensity and duration of saline stress, crop management and irrigation, and edaphoclimatic conditions (Tester and Davenport, 2003). The osmotic adjustment is highly important for the development of plants in a saline medium and any flaw in this equilibrium causes damages very similar to those by drought, such as loss of turgidity and decrease in growth and production (Ashraf and Harris, 2004).

The importance of nutrition supplementation with nitrogen (N) in plants under saline stress should also be taken into account. In fact, the macronutrient N has relevant physiological functions on the formation of organic compounds, with special reference to amino-acids, proteins, coenzymes, nucleic acids, vitamins, chlorophyll and others (Chaves et al., 2011). Further, Silva et al. (2008) report that N fertilization enhances plant growth and decreases the deleterious effects of high salt concentration on the growth and production of crops. In fact, the accumulation of these solutes (glycine betaine, N-amino solutes, proline; lysine, glutamine, aspartic acid), increases the capacity of osmotic adjustments of plants to salinity and tolerance of crop to water and saline stress.

Current research assesses the effects of irrigation with water containing different salinity levels and N doses on N, P and K contents in the castor bean plant cv. BRS Energia.

MATERIALS AND METHODS

The experiment was conducted under the field condition in an experimental area of Agricultural Sciences and Food Technology Center of the Federal University of Campina Grande (CCTA/UFCG) in Pombal, Paraíba - Brazil, at 6°48'16" S and 37°49'15" W, average altitude 144m, during September 2011 and January 2012 using plasticcontainers as drainage lysimeters. Five levels of electrical conductivity of irrigation water (ECw = 0.3; 1.2; 2.1; 3.0 and 3.9 dS m^{-1}) were used combined with four N rates (70; 100; 130 and 160 mg N kg^{-1} of soil). These doses were based on the recommendations of Novaiset al. (1991) for assays in pots (100 mg kg^{-1}). Experimental design consisted of randomized blocks (5x4), with three replications. The experimental units were distributed in simple rows spaced 0.90 m apart, with 0.70 m between plants in the row.

The seeds were sown in the 100 L plastic containers. After thinning, one plant per pot was kept and formed the experimental unit. Each recipient had two holes in its lower segment for drainage of excess water and plastic bottles for the collection of drained water were placed beneath the holes to estimate water consumption per plant. Recepients were filled with 2 kg of pebbles number zero which covered the bottom of the recipient. Further, 107.5 kg of Eutrophic Entisol of sandy loam texture, non-saline and non-sodic, collected at a depth between 0 - 30 cm, were placed on the pebbles. Soil, retrieved from the municipality of Pombal, PB - Brazil, was ground and sieved (<0.02 mm)and its physical and chemical attributes (Table 1) were analysed by methodology described by Claessen (1997).

Fertilization was performed for potassium and phosphorus based on the recommendations of Novais et al. (1991), using 12.0 g of potassium chloride and 162.5 g of monoammonium phosphate. In

Table 2. Anova results for N, P and K contents in different tissues of the castor bean plant after 120 days of sowing irrigated with water of different salinity and N rates.

Source of variation	DF	Mean square								
		N			P			K		
		Leaf[1]	Stem[1]	Root[1]	Leaf	Stem	Root[1]	Leaf	Stem	Root[1]
Salinity levels (ECw)	4	11.57ns	6.88**	3.58**	0.31ns	0.99*	1.34*	165.20**	123.04**	8.22*
Linear regression	1	18.54ns	18.42**	0.13*	0.14ns	1.21*	4.03**	11.19ns	400.66**	2.22ns
Quadratic regression	1	26.44ns	4.36**	2.58**	0.14ns	0.49ns	0.06ns	548.78**	25.88**	17.42*
N doses (N)	3	12.15ns	0.74*	1.65*	2.76*	1.17*	0.28ns	55.00*	12.36*	1.56ns
Linear regression	1	14.66ns	1.67*	4.94*	7.71*	1.35*	0.25ns	148.78*	22.20*	3.21ns
Quadratic regression	1	3.47ns	0.42ns	0.01ns	0.02ns	0.22ns	0.20ns	6.35ns	6.14*	0.90ns
Interaction (ECwx N)	12	9.31ns	0.23ns	0.90*	1.06ns	0.78*	0.16ns	19.78ns	0.29**	2.40ns
Residue	38	6.64	0.26	0.37	0.64	0.34	0.23	11.74	1.78	1.30
CV (%)		10.68	18.41	14.11	18.29	19.88	13.39	14.64	14.57	11.57

(*) and (**) significant at 0.05 and 0.01 probability, respectively; (ns) not significant; [1]statistical analysis after data transformation in $\sqrt{X}$; DF – Degrees of freedom.

addition 2.5 kg of vermicompost (6.3 g N kg^{-1}; 1.28 g P kg^{-1}; 0.53 g K kg^{-1}) was also applied to increase fertility and water retention capacity of soil.

Nitrogen was applied in split doses, one third was applied at the start of the experiment and the other two thirds in four equal applications along with irrigation water at 10-day intervals starting 25 days after sowing (DAS). In treatment N2 (equivalent to 100% recommendation), 33.34 g mono-ammonium phosphate (MAP) and 8.88 g urea were applied. Two foliar applications with Albatroz (N - 10%; P_2O_5 - 52%; K_2O - 10%; Ca - 0.1%; Zn - 0.02%; B - 0.02%; Fe - 0.15%; Mn - 0.1%, Cu - 0.02%; Mo - 0.005%) in the proportion of 1 g L^{-1}of water, at the start of flower emission (29 and 37 DAS), were also undertaken.

Considering the contents of N, P and K found in the vermicompost and the albatroz solution treatments received additional amounts of 6390, 21900 and 1070 mg kg^{-1} of N, $P_2O_5$and K_2O, respectively, however, these additional amounts on top of recommended dosages of Novais et al. (1991) for nitrogen, phosphorus and potassium were not considered for analyzing the effect of N since all treatments had received the same amount of vermicompost and albatroz solution and the same being negligible compared to pre-established doses of N.

Different salinity levels were obtained by addition of NaCl in water from the local supply system and quantity was determined by employing relation C (mg L^{-1}) = 640 × ECw (dS m^{-1}), (Rhoades et al., 2000). After soil conditioning in the recipient, water content was raised to field capacity by capillary saturation, followed by free drainage with the respective type of water. After preparation and ECw calibration, using a portable conductivity meter, the waters were stored in plastic recipients, adequately protected in order to avoid evaporation. The subsequent irrigations occurred daily at 17.00 h and volume of water to be applied was calculated according to irrigation requirements determined by water balance, or rather, volume applied minus volume drained in the previous irrigation.

The contents of N, P and K in leaf, stem and root were assessed after 120 DAS. The plants were harvested and their respective parts separated and dried in a forced air oven at 65°C till constant weight. The plant samples were then weighed, milled and analyzed, following methodologies recommended by Silva (1999). Data were evaluated by analysis of variance with F-test at 0.05 probability using SISVAR-ESAL (Ferreira, 2003) and regression analysis were performed when effects were significant. Choice of model of

regression was based on value of coefficient of determination (R^2) taking into consideration a plausible biological explanation.

RESULTS AND DISCUSSION

Analysis of variance (Table 2) showed that levels of water salinity (ECw) had a significant effect on N and P contents in stem and root tissues, whereas K contents of all organs of the castor bean plants under analysis were significantly influenced by this treatment. For N rates, there was a significant effect on N accumulation in stem and root tissues. Nitrogen rates also affected (p<0.05) P and K contents in leaf and stem tissues. The interaction between ECw *versus* N rates was significant for concentrations of N in the roots and P and K in the stem tissues, respectively.

The foliar N content showed a mean value of 11.98 g kg^{-1} which was not affected by salinity in irrigation water, but there was a 67.71% increase in N content of stem with per unit increase (p<0.01) of ECw (Figure 1a). Results for N content in the stem corroborate with that of Al-Harbi (1995) who reported the non-dependence between N contents and salinity levels for the period in which the plant underwent stress and especially for the species and genotypes with different salt tolerance.

The N content in the roots showed a quadratic response (p < 0.05) for plants with doses of 70 and 100 mg kg^{-1} N (Figure 1b). However, there was no significant effect for doses of 130 and 160 mg Nkg^{-1}, respectively, which showed average contents of 2.45 and 2.66 g Nkg^{-1}. Decrease in stem and root N contents in plants submitted to NaCl stress may have been caused by the competition between chloride (Cl$^-$) and nitrate (NO$_3^-$) ions transporters (Ogawa et al., 2000) and/or the inactivation of NO$_3^-$ transporters due to the toxic effects of the ions (Lin et al.,
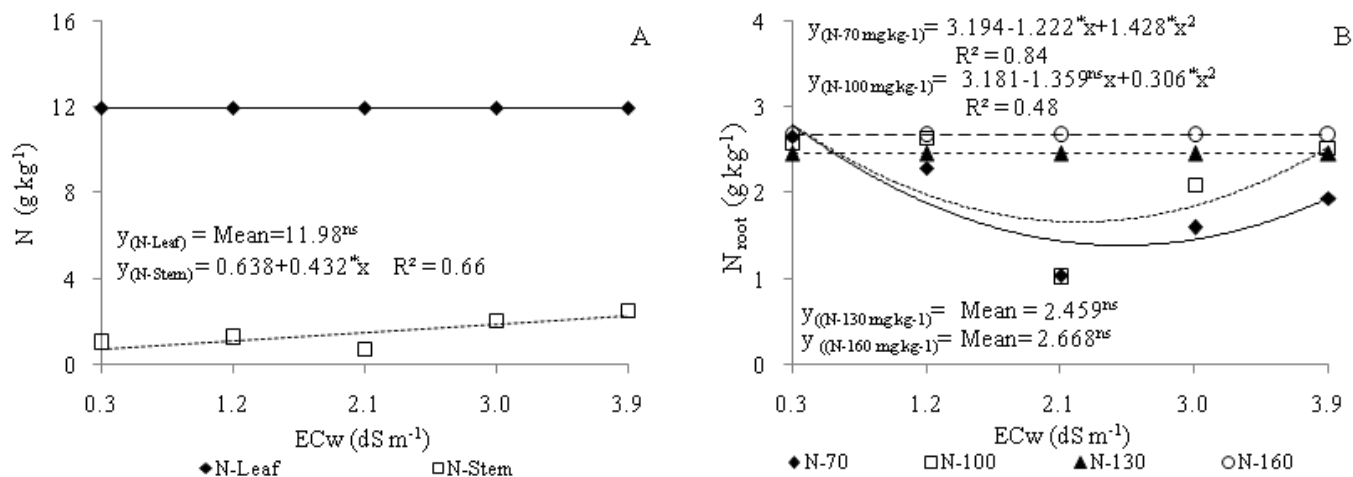
Figure 1. N content in stem as a function of electrical conductivity of irrigation water - ECw (A), and N content in roots (N_{root}) as a function of the interaction between ECw and N rates (B), 120 days after sowing (DAS).
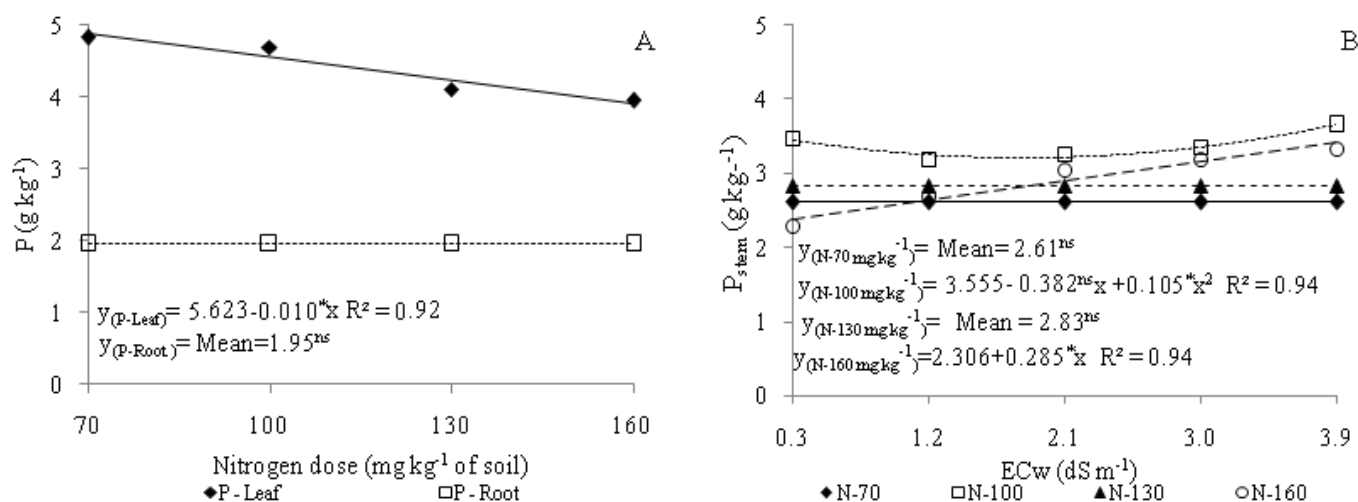

Figure 2. Phosphorus content in leaf (P_{leaf}) as a function of N fertilization (A), and P content in stem (P_{stem}) according to the interaction between electrical conductivity of irrigation water –ECw and N doses (B), 120 days after sowing (DAS).

2002). Salinity also causes the rupture of the root membrane and inhibits NO_3^- absorption (Parida and Das, 2004).

Further, saline stress interferes in enzymes similar to ammonium (NH_4^+), with a greater accumulation of the ion in the tissue, besides the accumulation from the degradation of proteins caused by the stress (Zhou et al., 2004). Costa et al. (2008) also reported that increase of salt concentration of the irrigation water (0.15 to 4.5 dS m^{-1}) caused deleterious effect on N concentration in root tissues of *Amaranthus* spp.

Phosphorus content in leaf tissues was negatively affected by N doses (Figure 2a). There was a 5.33% decrease in P for each interval of 30% in N doses. When

plants were submitted to the maximum N dose (160 mg kg^{-1}), P content in leaves decreased by 0.9 g kg^{-1} (16%) when compared to those with a lower dose (70 mg kg^{-1}). According to Malavolta et al. (1997), P is a highly mobile element in plants and reaches the leaves or the meristhematic locations by long distance transport and together with N is immediately distributed in the above ground plant material. With the aging of leaves, up to 60% of P may also be transported by the phloem to other segments of the plant, especially to new organs and developing fruit, with a decrease in P content of the leaves.

There was a quadratic response of plants with 100% N dose with regard to P contents in stem tissues (Figure
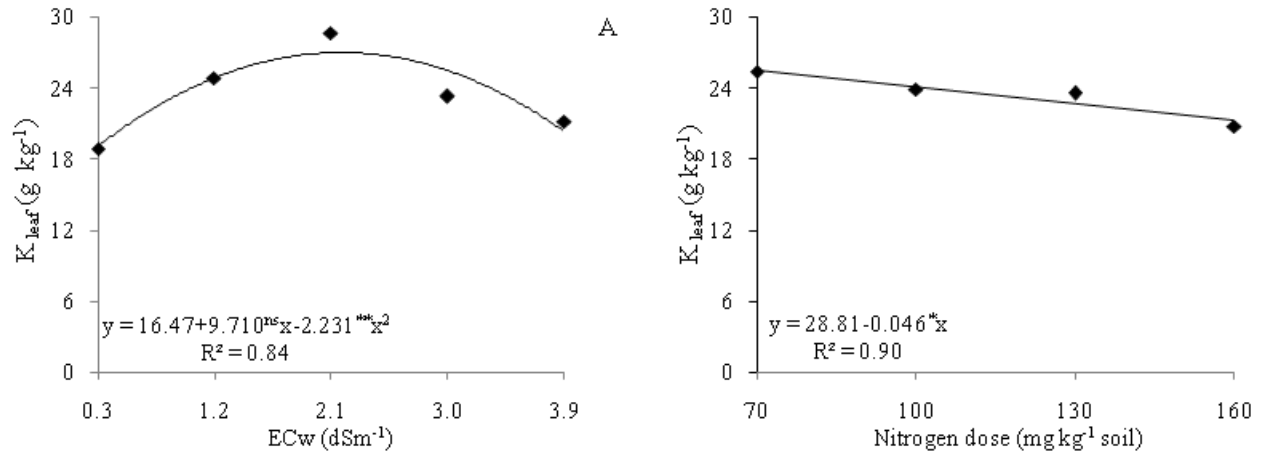
Figure 3. Potassium contents in leaf as a function of the electrical conductivity of irrigation water -ECw (A) and N rates (B), 120 days after sowing (DAS).

2b). High accumulation of P (3.65 g kg^{-1}) was reported under water salinity of 3.9 dS m^{-1}. However, N dose of 160 mg kg^{-1} caused a linear increase in the concentration of P in the stem, with a 12.35% increase with per unit increase in ECw. There was a 44.49% (1.02 g kg^{-1}) increase in P amounts in plants submitted to ECw=3.9 dS m^{-1} when compared to plants irrigated with lower salinity level (0.3 dS m^{-1}). It may be observed (Figure 2b) that the application of doses of 70 and 130 mg kg^{-1} N did not affect P content in the stem tissues of the castor bean plant but registered mean concentrations of 2.61 and 2.81 g kg^{-1} P, respectively.

Several studies demonstrated that the interaction between water salinity and P uptake in plants is a highly complex issue and depends on plant species, salt concentration, type of salt and salinity level of irrigation water (Grattan and Grieve, 1999). However, the accumulation of P in the stem of plants under salt stress may be a consequence of the decrease of translocation associated with a possible reduction in growth rate (Lacerdaet al., 2006). Results corroborate those observed in gliricidia (Farias et al., 2009) and cowpea (Silva et al., 2011) plants cultivated under saline stress.

Plants irrigated with ECw=2.2 dS m^{-1} caused a high accumulation of K contents in leaves (27.03 g kg^{-1}) with a decrease in ion concentration above this ECw level (Figure 3a). The low uptake of K in plants under high salinity levels has been attributed to the competition between Na$^+$ and K$^+$ by the absorption sites of the plasmalema and to the great efflux of K$^+$ from the roots as a direct result of osmotically induced exchanges in the permeability of the plasmalema and the substitution of Ca^{2+} by Na$^+$ in the membrane (Ferreira et al., 2001).

The first explanation is mainly due to the fact that the two cations (Na$^+$ and K$^+$) have a similar atomic radius and K$^+$ transporters are less specific in toxic concentrations of Na (Castillo et al., 2007). Results in current research confirmed results obtained by Sousa et al. (2012) when

they evaluated the influence of ECw in macro-and micronutrient contents in leaves of *Jatropha curcas*. Authors reported a quadratic effect and a highest K content (32.63 g kg^{-1}) in plants under ECw=2.2 dS m^{-1}.

With regard to the effect of N dose on the K content of leaves (Figure 4b), there was a decrease of 4.79% in the concentration of K$^+$ with each 30% increase in the N dose, which amounts to a decrease of 14.37% of K when data of plants cultivated under the highest (160 mg kg^{-1}) and lowest N dose (70 mg kg^{-1}) were compared. According to Rosolem (2005), K is a strong competitor with other cations owing to the high efficiency of the plants´ absorption system. Absorption of the other cationic ions increases when K$^+$ is absent in the solution since competition is less. The divergences in results in current study and those observed by Pacheco et al. (2008) may be explained considering that the latter studied the effects of NPK fertilization on castor bean IAC 226 and reported high contents of K$^+$ when combined to high N doses.

The interaction between salinity and N dose interfered in the accumulation of Kin the stem tissues of the castor bean plant (Figure 4a), showing a quadratic response for all N doses. Maximum K$^+$ contents (13.57, 11.41, 11.87 and 11.21 g kg^{-1}) occurred when the plants underwent irrigation with ECw of 3.9, 3.3, 3.9 and 3.1 dS m^{-1}, respectively. The lowest content of the nutrient (4.84, 6.13, 5.50 and 4.10 g Kkg^{-1}) occurred in plants irrigated with water at ECw=0.3 dS m^{-1}for N rates of 70, 100, 130 and160 mg kg^{-1}, respectively. Selectivity increase of the K membrane is relevant to reduce the effect of salinity in plants since it is the main ion in osmotic adjustment and in the maintenance of cell turgor (Munns, 2002).

Potassium content was also significantly affected by the interaction of water salinity of irrigation water and N doses (Table 2). Figure 4b shows that plants with 70 and 100 mg kg^{-1} of N decreased quadratically, with maximum contents of K (6.89 and 7.36 g kg^{-1} respectively), obtained
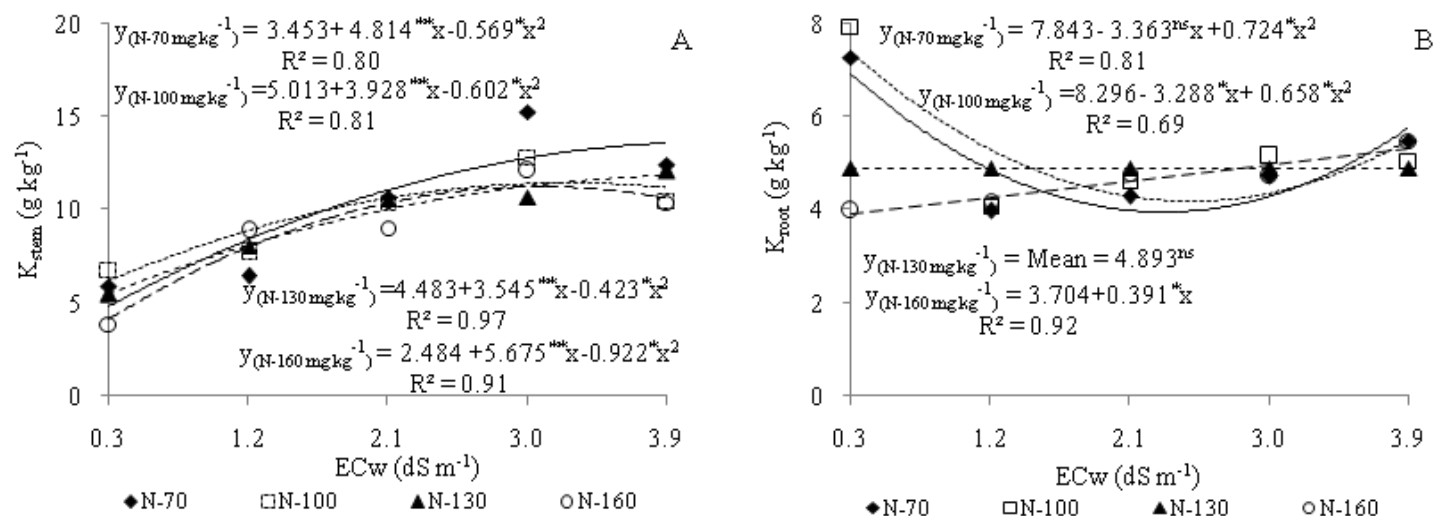
Figure 4. Potassium contents in the stem (K_{stem}) (A) and root (K_{root}) (B) as a function of the interaction between the electrical conductivity of irrigation water (ECw) and N rates, 120 days after sowing.

in plants irrigated with ECw of 0.3 dS m^{-1}. There was a growing linear response with dose 160mg kg^{-1}N which caused a 10.3% increase in K content with per unit increase in ECw (Figure 4b) or K content in plants irrigated with ECw=3.9 dS m^{-1} increased by 1.40 g kg^{-1} (37.10%) when compared to plants under ECw=0.3 dS m^{-1}. There were no significant statistical effects for dose of130 mg kg^{-1} N, averaging 4.89 g kg^{-1} K in the roots (Figure 4b).

Decrease in K content in the root is possibly due to the direct exposure of roots to saline solution which causes changes in the integrity and selective permeability of the plasma membrane (Grattan and Grieve, 1999). Viana (2007) analysed the combination of N and K doses in wheat, in a controlled area, and reported that K concentration in the dry matter of the roots decreased as N supply increased.

Conclusions

Highest potassium content in root and leaf tissues are obtained under irrigation water salinity of 0.3 and 2.2 dS m^{-1}, respectively. The N content in leaves of castor bean is not affected by levels of salinity in irrigation water and N doses tested. The application of nitrogen doses causes decrease in leaf P and K contents.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Milk production function and resource use efficiency in Jaipur District of Rajasthan

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The study was undertaken during 2013 and 2014 in Jaipur District of Rajasthan with the objectives to examine the input-output relationships and assess the resource use efficiency in milk production. The study covered 100 commercial dairy farms. The results of Cobb-Douglas production function revealed that expenditure on dry fodder and green fodder for small category of herd size were found to be positive and significant. The results for medium category of herd size revealed that partial regression coefficients for expenditure on green fodder and miscellaneous expenditure for were found positive and significant. The results for large category of herd size revealed that the partial regression coefficient for expenditure on concentrate and miscellaneous expenditure were found positive and significant. In case of small category of herd size, it was observed that dry fodder, green fodder, labour and miscellaneous expenses were optimally utilized. In case of medium category it was observed that dry fodder, green fodder and miscellaneous expenses were optimally utilized. In case of large category of herd size, dry fodder, concentrate and miscellaneous expenditure were optimally utilized.

Key words: Concentrate, dry fodder, green fodder, labour, milk, mvp and resource use efficiency.

INTRODUCTION

One of the most significant changes in India's agricultural economy over the past three and a half decades has been the rising contribution of livestock sector in the agricultural gross domestic product. Between 1970 and 2012, the share of livestock in agricultural gross domestic product has risen from 17 to 26% (Govt. of Rajasthan, 2014). The milk production is influenced by various genetic and non-genetic factors. The non-genetic factors influencing the milk production are quantity and quality of feeds and fodders fed, order of lactation, stage of lactation, herd size, labour use etc. Hence the selection

of suitable variables to study the milk production is very essential. To ensure the optimal use of various inputs used by the milk producers is matter of primary concern. It is important to know whether the inputs owned by commercial dairy farmers are used efficiently or not. An empirical assessment of determinants of milk production and resource use efficiency are important for planning, projecting and formulating dairy development policies in a particular region of the country. The input-output relationship in milk production and resource use efficiency have been studied by several researchers in

the various parts of the country and found different for different areas depending upon the type of milch animals and the milk production technology. No study has been carried out to investigate the milk production function and resource use efficiency in respect of commercial dairy farms in Jaipur district of Rajasthan.

The present study was undertaken to fill this vital gap with the specific objectives to (i) examine the input-output relationships in milk production across different categories of herd sizes of commercial dairy farms, and (ii) Study the resource use efficiency in milk production across different herd size categories of commercial dairy farms.

REVIEW OF LITERATURE

Mahajan (2010) in his study economic analysis of rural and pere urban farm in Ludhiana district of Punjab indicated that Cobb Douglass function was best fit. In production function of peri urban dairy farm, the partial regression coefficient of expenditure on concentrate for crossbred cattle were found positive and significant with total explained variation, that is, R^2 as 84.3%.

Singh (2008) in his study on economic analysis of milk production of milk production in Varanasi District of Utter Pradesh concluded that green fodder; dry fodder and concentrate were underutilized indicating that feeding of more quantity of green fodder and concentrate will further increase the productivity of milch buffaloes in the study area.

Wani et al. (1992) studied input-output relationship in milk production and estimated the marginal value product of relevant input variables separately for non descript and crossbred cows in the Kashmir valley.

METHODOLOGY

The study was conducted during 2013 to 2014 in Jaipur district of Rajasthan. The sampling design consisted of selecting the ultimate sampling unit, that is, dairy commercial farms using multistage random sampling method. The tehsils, villages and sample commercial dairy farms consisted the first, second and third stage of sampling. Jaipur district has 13 tehsils namely Amer, Chomu, Jamwaramgarh, Phagi, Phulera, Bassi, Sanganer, Muzamabad, Viratnagar, Kotputli, Chaksu, which were classified on the basis of water and type of animal into different zones. Out of thirteen tehsils, six tehsils namely Amer, Bassi, Shahpura, Sanganer, Phuleraand Jaipur thesil were selected consisting of 590 commericial dairy farms. Among 590 commercial dairy farms 100 commercial dairies were selected on the basis of probability proportional to size.

The selected 100 commercial dairies were post stratified into three categories using Cumulative Square Root Frequency Method on the basis of number of milch animals. The commercial dairy farms were thus categorized into three herd size categories namely small (up to 31 milch animals), Medium (32-45 milch animals) and large (above 45 milch animals). The distribution of sampled commercial dairy farms in the small, medium and large herd size categories were found to be 43, 41 and 16 respectively. The primary data of commercial dairy farms were collected with help of well structured pre-tested schedule by personal interview/enquiry method. The data were collected on milk production, quantity of green fodder, dry fodder, concentrate and miscellaneous expenditure along with their monetary values.

Analytical framework

Specification of milk production function

The specification of milk production function used in the present study for functional analysis is as follows:

$Y=f (X_1, X_2, X_3, X_4, X_5)$

Where, Y = Income from milk per farm per day (Rs.); X_1 = Expenditure on green fodder per farm per day (Rs.); X_2 = Expenditure on dry fodder per farm per day (Rs.); X_3 = Expenditure on concentrate per farm per day (Rs.); X_4 = Value of labour used per farm per day (Rs.); X_5 = Miscellaneous expenses per farm per day (Rs.).

Four types of functional forms, viz., Cobb-Douglas, Linear and Semi log (both linear-log and log-linear models) were tried which are as follows:

Linear:
$$Y = a + \sum_{i=1}^{n} bi\, Xi + \mu$$

Cobb Douglas:
$$Y = a \prod_{i=1}^{n} X_i^{bi}\, e^{\mu}$$

Semi Log(Log-Lin):
$$lnY = a + \sum_{i=1}^{n} b_i\, X_i + \mu$$

Semi log(Lin-Log):
$$Y = lna + \sum_{i=1}^{n} b_i\, lnX_i + \mu$$

Where, Y = value of output; X_i = value of ith input used; a = constant term; b_i = partial regression coefficient of the ith input to be estimated; μ = random error distributed normally with zero mean and constant variance, and e = base of natural log.

The best function will be selected on the following economic and statistical criteria:

1. The higher value of coefficient of multiple determination (R^2)
2. Significant level of individual regression coefficients, and
3. The ability of the function to provide economically meaningful results.
4. The minimum value of Root Mean Square Error (RMSE).

Cobb-Douglas production function was found best fit for all categories of commercial dairy farms because it has high value of R^2 and low value of Root Mean Square Error (RMSE) among all other fitted production functions (Table 1).

Ideally, the output (Y) and inputs (X_i) in the above production functions were measured in monetary values rather than their

Table 1. Root mean square error (RMSE) and R^2 (%) values of different fitted production functions.

| Type of function | Category of commercial dairy farm | | | | | |
| | Small | | Medium | | Large | |
	RMSE	R^2 (%)	RMSE	R^2 (%)	RMSE	R^2 (%)
linear	0.629	23.520	18.180	84.360	0.123	87.780
log lin	0.253	21.910	0.062	84.760	0.047	87.790
Cobb-Douglas	0.235	32.420	0.066	82.740	0.043	89.630
lin log	0.586	33.260	0.197	81.600	0.114	89.600

Table 2. Various fitted production function and their marginal value product

Type of function	Marginal value product
Linear	b_i
Cobb Douglas	$b_i \times \dfrac{\bar{Y}}{\bar{X}}$
Semi log (lin log)	$\dfrac{b_i}{\bar{X}}$
Semi log (log lin)	$b_i \times \bar{Y}$

b_i = partial regression coefficient of the ith input to be estimated; $\bar{Y}$ = geometric mean of output Y, and $\bar{X}$ = geometric mean of input X

physical quantities. This was done because the quality of different feeds and fodders differ from one respondent to the other and can be more appreciably reflected in value terms. The monetary values of inputs in production functions have been preferred over physical quantities by many of earlier researchers e.g. Sharma and Singh (1993), Shiyani and Singh (1996) etc.

Marginal value product

Marginal value productivity of inputs was estimated from the fitted production function (Table 2).

Recourse use efficiency

Recourse use efficiency of an input measures whether or not the input is used optimally. The inputs are used optimally if the MVP of the input is equal to its price, that is,

$MVP_i = P_i$

Where P_i is the unit price of input
   In order to examine the resource use efficiency, the marginal value productivity of various inputs was worked out for significant regression coefficients in the estimated milk production function. Any deviation of MVP of input from its unit price may be termed as resource use efficiency. The higher the difference between MVP of an input and its unit price, the higher is the resource use inefficiency and vice versa.
   Further, t-statistics given below was used to test the statistical significance of the difference between MVP and its unit price. If the

difference between MVP of an input and its unit price is statistically non significant, it indicates that the inputs is being utilized efficiently. A significant higher MVP of an input than its unit price shows that the input can be used further to increase productivity, while a significantly lower MVP of an input is being used in excess and hence needs reduction.

t statistic:

$$t = \frac{MVPx_i - Px_i}{S.\,E.\,(MVPx_i)}$$

S.E (MVP_i) = Standard error of MVP, and P_i is the unit price of input.

RESULTS AND DISCUSSION

The Cobb-Douglas production function for all categories of commercial dairy farms has been presented in Table 3. A close perusal of the table revealed that the coefficient of multiple determination (R^2) for the small, medium, large and overall category was 32.42, 82.74, 89.63 and 17.73% of total variation in income from milk per farm per day, respectively, were explained by the variables included in the selected regression model.
   It was revealed from Table 3 that partial regression coefficients of expenditure on dry fodder and green

Table 3. Root mean square error (RMSE) and R^2 (%) values of different fitted production functions.

| Type of function | Category of commercial dairy farm | | | | | |
| | Small | | Medium | | Large | |
	RMSE	R^2 (%)	RMSE	R^2 (%)	RMSE	R^2 (%)
linear	0.629	23.520	18.180	84.360	0.123	87.780
log lin	0.253	21.910	0.062	84.760	0.047	87.790
Cobb-Douglas	0.235	32.420	0.066	82.740	0.043	89.630
lin log	0.586	33.260	0.197	81.600	0.114	89.600

Table 4. Estimated coefficients of milk production function for different categories of commercial dairy farms.

| Category | No. of dairy farms | Constant | Regression Coefficients | | | | | R^2 (%) |
			G.F (X_1)	D.F (X_2)	Conc. (X_3)	Labor (X_4)	Misc. (X_5)	
Small	43	2.571 (1.977)	0.155* (0.062)	0.487** (0.151)	0.736 (0.410)	-0.499* (0.229)	-0.293* (0.121)	32.42
Medium	41	3.100 (1.074)	0.631** (0.152)	-0.086* (0.136)	0.0966 (0.303)	0.191 (0.097)	0.297* (0.124)	82.74
Large	16	-8.967 (3.629)	-0.200** (0.022)	0.185 (0.281)	3.986*** (0.765)	-1.655** (0.417)	1.188** (0.240)	89.63
Overall	100	3.423 (1.193)	0.043 (0.083)	0.112 (0.099)	0.605* (0.242)	-0.344** (0.113)	-0.009 (0.083)	17.73

Figures in parenthesis indicate the standard error of regression coefficient. *Significant at $p < 0.05$, * significant at $p < 0.01$.

fodder for small category of herd size were found to be positive and significant. The production function analysis indicated that milk production could be increased through effective feeding of concentrates. The table further revealed that partial regression coefficients for labour and miscellaneous expenditure were negative and significant. The concentrate, was, thus, found to have no impact on income from milk.

The partial regression coefficients for expenditure on green fodder and miscellaneous expenditure for medium category of herd size were found to be positive and significant with total explained variation, that is, R^2 as 82.74%. The production function analysis indicated that milk production could be increased through effective feeding of green fodder and miscellaneous expenditure.

The partial regression coefficient for expenditure on concentrate and miscellaneous expenditure for large category of herd size were found to be positive and significant. The production function analysis indicated that milk production could be increased through effective feeding of concentrate and by increasing miscellaneous expenditure. The table further revealed that partial regression coefficient for labour and green fodder, it was negative and significant which implied that by increasing expenditure on green fodder and labour income from milk production will decrease. The partial regression coefficient for dry fodder, was, thus, found to have no

impact on income from milk. The study is similar to Mangesh (2003).

The partial regression coefficients for expenditure on concentrate and labour for overall were found to be positive and significant which implies that income from milk production could be increased through effective feeding of concentrate and by decreasing use of labour. The table further revealed that partial regression coefficients for rest of variables were found statistically non-significant which implied that these variables not have impact on income from milk. The study is similar to Das (2004)

In order to find out whether or not the significant inputs viz. green fodder, dry fodder, labor and miscellaneous in case of small category of herd size and green fodder, dry fodder and miscellaneous expenditure in case of medium herd size and green fodder, concentrate, labour and miscellaneous in case of large category, concentrate and labour in case of overall farm were used efficiently, Marginal value of productivities (MVP) of these inputs has been worked out (Table 4).

Resource use efficiency in milk production

In order to examine the resource use efficiency, the marginal value productivities (MVP) of inputs whose

Table 5. Resource use efficiency in milk production.

Small category	MVP	Input Price	Difference	S.E	t-value
Dry fodder	1.51	1.00	0.51	0.06	0.34
Green fodder	7.29	1.00	6.29	0.15	0.86
Labour	2.69	1.00	1.69	0.23	0.63
Miscellaneous	17.66	1.00	16.66	0.12	0.94
Medium category					
Dry fodder	3.57	1.00	2.57	0.15	0.72
Green fodder	7.65	1.00	6.65	0.14	0.87
Miscellaneous	23.25	1.00	22.25	0.12	0.96
Large category					
Dry fodder	0.57	1.00	-0.43	0.15	-0.11
Concentrate	1.95	1.00	0.95	0.30	1.23
Labour	8.14	1.00	7.14**	0.10	3.78
Miscellaneous	49.22	1.00	48.22	0.12	1.89
Overall					
Dry fodder	1.03	1.00	0.03	0.80	0.03
Green fodder	5.66	1.00	4.66	4.87	0.96
Concentrate	1.44	1.00	0.44	0.58	0.76
Labour	-5.03	1.00	-6.03**	1.66	-3.62
Miscellaneous	-1.52	1.00	-2.52	14.22	-0.18

**Significant at $p < 0.01$.

regression coefficients were found statistically significant in estimated milk production function were compared with their acquisition cost, that is, marginal factor cost (MFC).

The inputs viz., green fodder, dry fodder, labour and miscellaneous expenditure in case of small category, green fodder, dry fodder and miscellaneous expenditure in case of medium, green fodder, concentrate, labour and miscellaneous in case of large and concentrate and labour in case of overall herd size category of commercial dairy farm were found to be statistically significant. The marginal value productivity of all the significant inputs was computed at their geometric mean level. The results of the different herd size categories are presented in Table 5 along with their prices. Since all the inputs were expressed in monetary terms in the production function, the acquisition cost of the inputs was taken as Re.1. The estimated marginal value productivity was, therefore, compared with unity to examine the resource use efficiency. t-statistic was used to test the significance of deviation of MVP of an input from its unit price. A significant higher difference of MVP of an input from its unit price shows that more of that input can be used to increase productivity, while a significant lower difference MVP of an input from its unit price indicates that the input is used in excess and needs curtailment. The marginal value productivity (MVP) of significant inputs for all categories of commercial dairy farms, their difference with unit price of respective inputs (MFC) and t-statistic are given in the Table 5.

In case of small category of herd size, it can be observed that marginal value productivity of dry fodder, green fodder, labour and miscellaneous expenses were found to be positive but statistically non-significant. Similar findings were also observed in case of medium category of herd size, it can be revealed that marginal value productivity of dry fodder, green fodder and miscellaneous expenses were found to be positive but statistically non-significant.

The marginal value productivity of dry fodder, concentrate and miscellaneous expenses were found to be statistically non-significant in case of large category of herd size while it was positive and statistically significant for labour. The marginal value productivity of labour was under utilized as the difference between its MVP and unit price was positive and significant in the study area.

The marginal value productivity of concentrate was found to be statistically non-significant in case of overall category of herd size. The marginal value productivity of labour was negative and significantly lower than their acquisition cost which indicated that labour was over-utilized in the study area.

Conclusions

The green fodder, dry fodder, labour and miscellaneous

expenditure were found to be statistically significant in case of small category of commercial dairy farm, green fodder, dry fodder and miscellaneous expenditure in case of medium, green fodder, concentrate, labour and miscellaneous in case of large and concentrate and labour in case of overall herd size category. The results of resource use efficiency indicated that none of the marginal value productivity of all inputs was statistically significant across and overall herd size category except labour in large category and overall herd size category in the study area.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Predicting grain yield of maize using drought tolerance traits

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Two experiments (field and pot) were conducted to evaluate the ability of partial least square regression (PLSR) using physiological and root traits to predict grain yield of maize. The genetic materials used for the experiments were six maize genotypes. Data was recorded on some growth, physiological and root traits. Data was analyzed using PLSR model of XLSTAT. There was a good prediction of grain yield of maize using phenological traits (R^2 = 0.99 and RMSE = 17.73). The model gave a good fit in predicting grain yield with Sammaz 14 having the best prediction. Prediction model of grain yield using root and seedling traits also gave a good fit (r^2 = 0.96). Sammaz 14 and TZE-COMP 5 had better fits. Prediction of grain yield of maize using some physiological traits of maize also produced a good fit (R^2 = 0.86 and RMSE = 90.94). Prediction accuracy for Sammaz 14 was higher than the other genotypes. The good fits observed for all the predictions indicates the ability and usefulness of PLSR in predicting grain yield of maize and this can reduce the time of breeding programs in developing maize varieties that are tolerant to drought.

Key words: Partial least square, maize, drought, root, and physiological traits.

INTRODUCTION

Maize is the third most important food grain for humankind after rice and wheat. It is mostly grown under rain-fed conditions and among the cereals, it is the second most susceptible to drought next to rice. Drought is a rising threat of the world. Most of the countries of the world are facing the problem of drought. It is the creeping disaster, slowly taking hold of an area and tightening its grip with time (Misra et al., 2002). Constitutive variation for root traits is an important adaptation under drought prone conditions (Nguyen et al., 2011). Water is an integral part of plant body and it plays an important role in growth initiation, maintenance of developmental process of plant life and hence has pivotal function in crop production. Drought stress has deleterious effects on the seedling establishment, vegetative growth, photosynthesis, root growth, anthesis, anthesis-silking interval, pollination and grain formation in maize crop (Aslam et al., 2012). Annual maize yield loss due to drought is estimated to be 15% in West and Central Africa and losses may be higher in the marginal areas where the annual rainfall is below 500 mm and soils are sandy or shallow (Edmeades et al., 1995). The effect of selection under stress on yield performance of genotypes under optimal conditions and vice versa has been an ongoing debate among plant breeders for decades. Secondary traits can improve the precision with which

Table 1. List of genotypes used for the study.

Entry	Genotype	Maturity
1	SAMMAZ 14	Late
2	SAMMAZ 29	Extra-Early
3	2009 EVDT	Early
4	2009 TZE-W	Early
5	TZE-COMP 5	Early
6	2009 TZEE	Extra-Early

drought tolerant genotypes are identified, compared with measuring only grain yield under drought stress. Secondary traits such as canopy temperature, stomata conductance, ears per plant and anthesis silking interval have been found to possess strong correlations with grain yield under drought conditions and have been used to select for higher levels of tolerance to drought (Badu-Apraku et al., 2011). Predictions of grain yield of maize using secondary traits that are of significance to drought tolerance have not been fully exploited in Nigeria.

Recently, partial least square regression (PLSR) has been employed for predicting plant biomass, LAI, nitrogen and Chlorophy ll concentration and density of wheat using reflectance measurement of wheat canopy (Hansen and Schjoerring, 2003). The PLSR has been widely used to assess N related indicators of crops in homogeneous areas (Nguyen and Lee, 2006; Soderstrom et al., 2010). Limited research has been conducted to estimate grain yield of maize fields with different growth stages and cultivars. In general, studies reported so far have confirmed that PLS appeared as one of the most efficient method in extracting and creating reliable models in wide range of fields (Nguyen and Lee, 2006).

Partial least square can easily treat data matrices in which each object is described by several hundreds of variables (Galadi and Kowalski, 1986; Haaland and Thomas, 1988). This technique can extract the relevant part of the information for the very large data matrices and produce the most reliable models compared to other calibration methods (Thomas and Haaland, 1990). Partial least square regression has been successfully applied to NIR spectral data for predicting soil nitrate (R^2 = 0.94–0.95) (Ehsani et al., 1999), sodium chloride content of commercial king and hot smoked salmon fish (R^2 = 0.82–0.85) (Lin et al., 2003), and several chemical components of sunflower seeds (R^2 = 0.90–0.96) (Fassio and Cozzolino, 2003). PLSR overcomes the problems of co-linearity and "over-fitting" compared to step linear regression analysis if optimally choosing a suitable number of principal components and deleting the noise bands (Herrmann et al., 2011). However, the small number of sampling may limit the number of latent variables in the PLSR model and reduce the calibration accuracy (Van Der Heijden et al., 2007). The objective of

this study was to assess the predicting power of the relationship between physiological, shoot, and root traits with grain yield of maize using partial least square regression using.

MATERIALS AND METHODS

Two experiments (Screen House and Field) were conducted in the 2014 wet season at the Research and Teaching Farm of Department of Agronomy, Bayero University, Kano Nigeria (Lat 11°58'N, Long 8°25'E and 475 m above sea level). The experimental treatments consisted of six maize genotypes of varying maturity groups (Table 1). The treatments were laid out in a Completely Randomized Design (CRD) and Randomized Complete Block Design (RCBD) for the screen house and field experiments, respectively each replicated three times. The screen house experiment was maintained for 30 days and fully irrigated using watering can. Two seeds were sown per plot and later thinned to one per pot. Fertilizer was split applied at planting and two weeks after sowing using recommended rates. Data collected for the pot experiment were; root length, root fresh weight, shoot fresh weight, fresh root shoot ratio, dry root weight, dry shoot weight and dry root shoot ratio. In the field experiment, a single row plot of 4 m long was used. Three seeds were sown at 40 cm × 70 cm intra and inter row spacing, respectively. The seeds were later thinned to two plants per stand. NPK 15-15-15 was split applied at 2 and 6 weeks after sowing. Data were recorded on plant height at maturity, ear height, days to anthesis, days to silking, plant aspect and number of leaves. Drought tolerance related data collected includes: stomatal conductance and leaf temperature using leaf porometer (Decagon Devices), canopy temperature using infrared thermometer, and Chlorophy ll content using SC1 Handheld SPAD meter from Konica Minolta.

Partial least squares regression

In many situations, when the number of variables (S) is much larger than the number of observations (N), and there is high co-linearity among variables, the usual methods for fitting regressions based on ordinary least squares are not adequate. In this situation, partial least squares regression seems to be a more appropriate alternative. Details of PLS theory (Helland, 1988) and its similarities to principal components regression and stepwise multiple linear regression are described in Aastveit and Martens (1986). A description of univariate and multivariate PLS and their algorithms was given in Vargas et al. (1998). Partial least square (PLS) regression was carried out using the PLS module of the XLSTAT software (Addinsoft, 2009) to predict grain yield from three set of data collected (root, agronomical and physiological).

Model quality

The performance of the model is measured by coefficient of determination of the model (R^2) and the root mean square error (RMSE) that is an indicator of the average error in the analysis expressed in original measurement unit (Kvalheim, 1987). The higher the R^2 and the lower the RMSE, the higher the precision and accuracy of the model to predict the grain yield.

RESULTS AND DISCUSSION

The prediction of grain yield of maize using plant height

Table 2. Prediction of grain yield of maize using phenological traits.

Variable	Yield
Intercept	4175.407
Days to anthesis	18.856
Days to silking	-3.974
Anthesis silking interval	47.534
Plant aspect a	-281.952
Plant aspect b	-649.283
Plant height	-0.204
Ear height	2.833
Leaf number	-137.618
R^2	0.99
RMSE	17.73
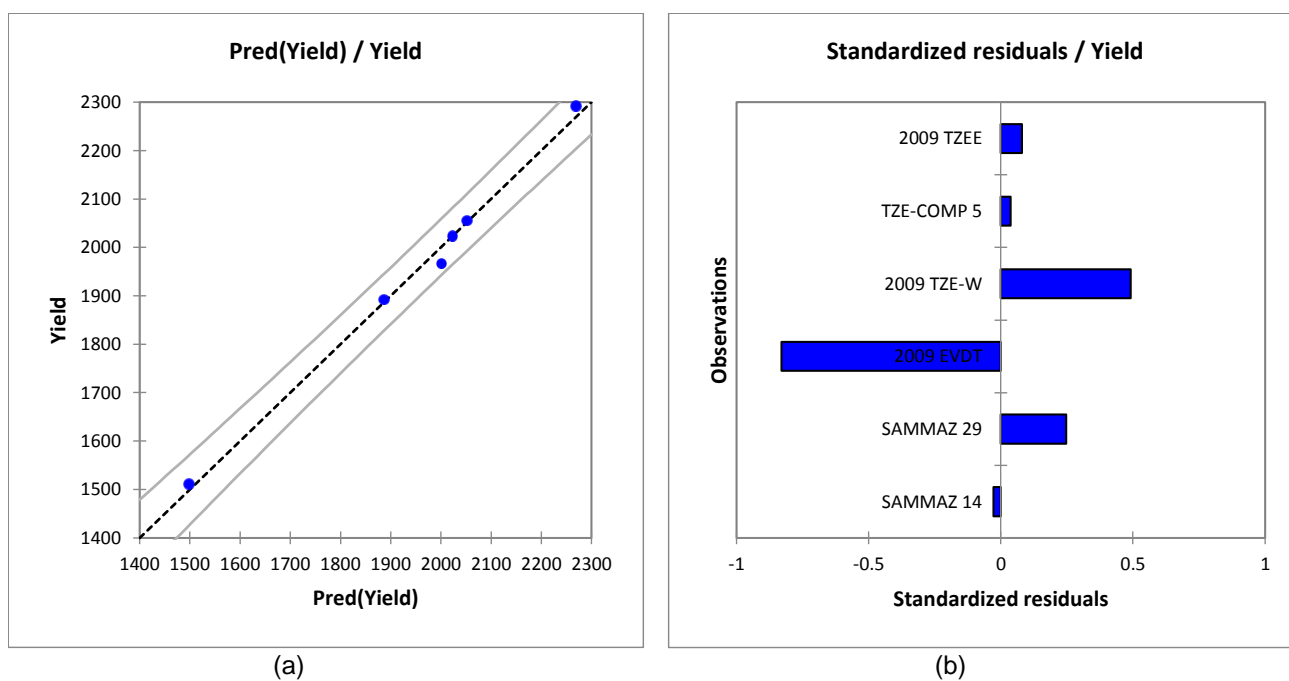
Figure 1. a) Plot of predicted yield against observed yield using phenological traits b) Standardized residual plots.

at maturity, ear height, days to anthesis, days to silking, plant aspect and number of leaves are presented in Table 2. The R^2 value was high (0.99) with a relatively low RMSE (17.73) indicating a good fit for yield prediction using these traits. From the Table, days to silking and plant aspect have negative contributions in the model. Anthesis silking interval had the highest positive value (47.534). Figure 1a also shows a good accuracy of prediction of grain yield for all the genotypes used. Grain yield of Sammaz 14 was predicted more accurately and had the least standardized residual (Figure 1b). The prediction of grain yield of 2009 EVDT and 2009

TZEW were fairly accurate because of the high residuals observed.

Table 3 shows the prediction of grain yield using root traits. The R^2 value (0.96) obtained indicates a good fit for prediction of grain yield. A low RMSE value was also obtained. Fresh root shoot ratio had the highest positive contribution in the model. A good fit was observed generally in all the prediction for the different genotypes (Figure 2a). However, Sammaz 29 and TZE-COMP 5 gave better fit in the prediction while Sammaz 14 and 2009 TZEW were fairly predicted (Figure 2b). Prediction of grain yield of maize using leaf temperature, canopy

Table 3. Prediction of grain yield of maize using root traits.

Variable	Yield
Intercept	8255.874
Dry root shoot ratio	-330.825
Dry root weight	11.227
Dry shoot weight	-7.946
Fresh root shoot ratio	260.936
Fresh root weight	-1.427
Fresh shoot weight	-12.642
Leaf number	-933.711
Root length	2.433
R^2	0.96
RMSE	44.16
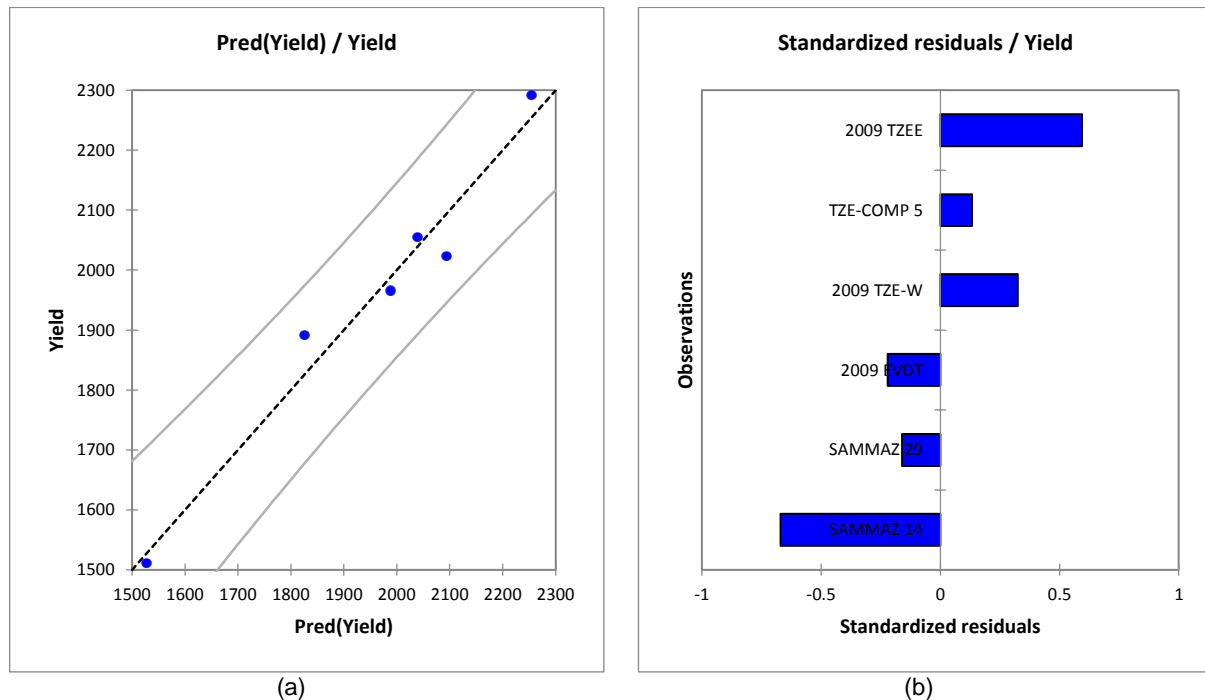
Figure 2. a) Plot of predicted yield against observed yield using root traits b) Standardized residual plots

temperature, stomatal conductance and Chlorophyll content are presented in Table 4. These traits had negative contribution to grain yield. The R^2 value was 0.85 with RMSE of 90.94, thus, indicating a fair accuracy of prediction. The grain yield of Sammaz 14 was better predicted than the other genotypes (Figure 3a and b).

Generally, there was a good fit for predictions using above ground traits. However, prediction using root traits was fairly accurate. This finding is similar to the report of Nguyen and Lee (2006), and Li et al. (2014) who reported a good fit for prediction of rice leaf growth and canopy nitrogen of wheat, respectively using PLSR. Sammaz 14

was accurately predicted when agronomic and physiological (drought related) traits were used. This may be attributed to the late maturity of the genotype (110 days) when compared to other genotypes that are either extra early or early maturing. However, in terms of prediction using root traits, Sammaz 29 was better predicted followed by TZE-COMP 5. Vargas et al. (1998) used the PLSR in interpreting the genotype by environment interaction of wheat and observed that the PLS was effective in detecting environmental and cultivar explanatory variables associated with factors that explained large portions of the interaction. Further

Table 4. Prediction of grain yield of maize using physiological traits.

Variable	Yield
Intercept	20371.068
Leaf temperature	-428.334
Canopy temperature	-70.626
Stomatal conductance	-0.775
Chlorophyll content	-17.001
R^2	0.85
RMSE	90.94
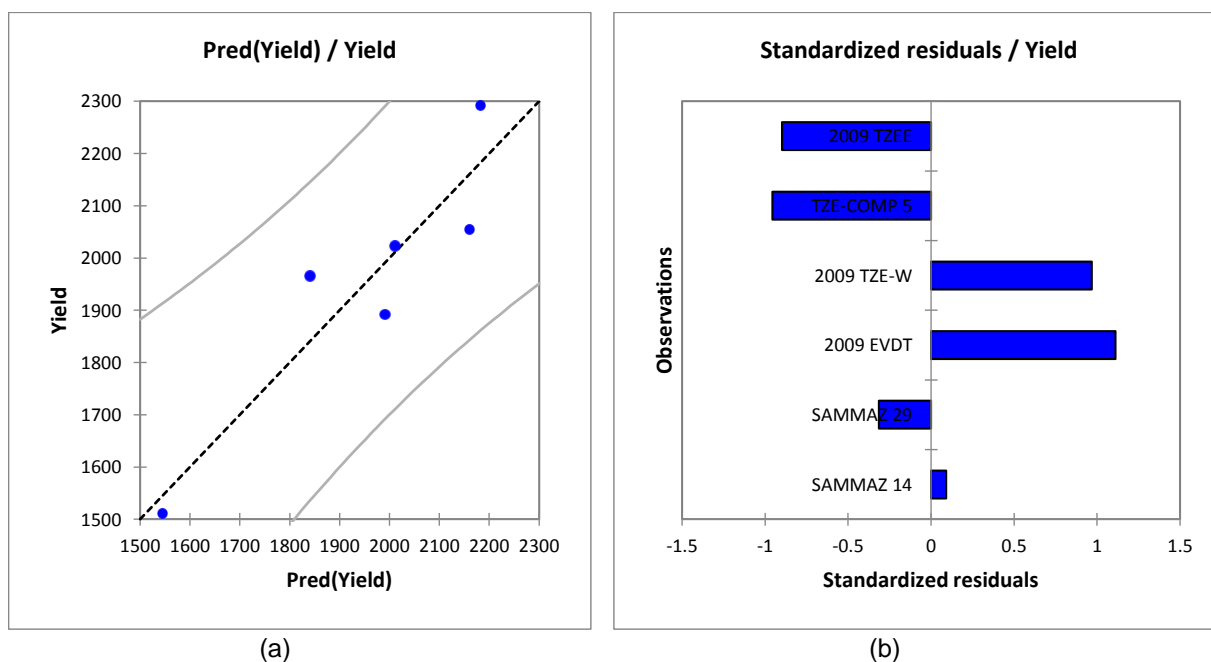
Figure 3. a) Plot of predicted yield against observed yield using physiological traits b) Standardized residual plots.

research is recommended to evaluate the predicting power of PLS using drought tolerant related traits that are less tedious to measure as this can enhance screening and selection of drought tolerant maize genotypes at early stages.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Deployment of indigenous wild *Ganoderma lucidum* for better yield on different substrates

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***Ganoderma lucidum* is a medicinal mushroom growing on angiospermous trees that has been used in the Orient medicine for more than 2000 years. Since now studies have been almost entirely limited to laboratory scale and much more understanding on the growth morphology and mass production is necessary to develop economical large scale processes. In this study, domestication of wild isolate of *G. lucidum* was done, which was collected from Himalayan region of India. Effect of various kinds of sawdust and bran on its yield was also investigated in artificial cultivation. Four kinds of sawdust viz. *Mangifera indica*, *Jacaranda mimosifolia*, *Populus alba* and *Dalbergia Sissoo* along with Coir Pith were used as a substrate media in *G. lucidum* cultivation. Significant differences (P<0.01) were found among varieties of sawdust, and mixtures, both in yield and biological efficiency (BE). The highest yield and BE were obtained from Oak sawdust and its combination with wheat bran.**

Key words: *Ganoderma lucidum*, cultivation, sawdust, bran, substrate, yield.

INTRODUCTION

Ganoderma lucidum (Fr.) Karst (Polyporaceae) has been recognized as a medicinal mushroom for over 2000 years and its powerful effects have been documented in ancient scripts. Besides promoting longevity, it has unique properties of strengthening the immune system[1]. It is a popular remedy to treat conditions like chronic hepatitis, hypertension, cancer, low blood pressure, high blood pressure, diabetes, rheumatism, heart problems, paralysis, ulcers, arthritis, asthma, tiredness, hepatitis A, B, and C, sterility, psoriasis, mumps, epilepsy and alcoholism (Zang, 1999). It refreshes the body and mind, delays ageing and is also effective in reducing the blood glucose level of diabetic patients after two months of treatment. Tribes in India have also been using the extract of this fungus for the treatment of joint pain (Harsh

et al., 1993). All parts of Reishi mushroom, such as spore, mycelium and basidiocarp are used for health purpose (Wasser, 2002).

The modern herbal industry and consumer demand genuine true-to-type *Ganoderma* which is mature and free from unwanted microbes and chemicals. The recent increase of interest in herbal medicines for various physiological disorders and recognition of many biological response modifiers (BRM) in *Ganoderma* products has attracted the attention of researchers globally. As a result, many countries have developed cultivation technology of this mushroom. Scientists have attempted artificial cultivation of Reishi first time in 1937 and mass production was achieved in 1971 by seeding the spawn in sawdust containing pots (Perumal, 2009). *G. lucidum* is

most commonly cultivated in China, Taiwan, Japan, Korea, Malaysia and North America. In India, Directorate of Mushroom Research, Solan, has achieved success in cultivating this mushroom employing various combinations of sawdust and other nutrients supplements and environmental conditions such as light, pH, temperature, humidity under laboratory conditions (Rai, 2003).

Global production of *Ganoderma* was about 4900 to 5000 tonnes in 2002, out of which 3800 tonnes were produced in China (Lai et al., 2004). The fruit body of *G. lucidum* is sold in the market at Rs 600-700/kg (Perumal, 2009). Its trade is also growing fast in India due to import from other countries.

Though *Ganoderma* is available in the tropics, temperate and subtropical regions, huge requirements of fruiting body and their spores cannot be met from wild collections. To make *G. lucidum* easily available to the general public around the year its cultivation under *in vitro* conditions is of paramount importance. Therefore, in this work cultivation of wild isolate under *in vitro* conditions was tried along with determination of best kind of substrate for its cultivation.

MATERIALS AND METHODS

The strain of *G. lucidum* was collected from the forest of Dhualadhar range in Himalayan region of India and identified by using conventional description method and molecular techniques. Culture was prepared on malt extract agar (MEA) medium by tissue culture from the basidiocarp. The mycelium from MEA slant was used for spawn production on wheat grains. Experimental cultivation was attempted on four locally available sawdust based substrates and Coir Pith. All the substrates were used in various combinations. Sawdust from different broad leaf tree species (*Mangifera indica*, *Populus alba*, *Jacaranda mimosifolia*, and *Dalbergia Sissoo*) from sawmills and Coir Pith were collected and evaluated in various combinations with bran such as (10% wheat bran+ 90% sawdust) and (20% wheat bran + 80% sawdust). Apart from various combinations 100% sawdust of above mentioned trees and 100% wheat bran were also evaluated. All substrates were wetted to 65% moisture level. Calcium sulphate (gypsum) and Calcium carbonate (chalk powder) were added to get a pH of 5.0.

Then the mixed substrates (700 g dry wt.; 1000 g wet wt.) were filled in polypropylene bags and plugged with non-absorbent cotton after putting a plastic (PVC) ring at the neck. For each treatment, four replications were maintained. The substrates were sterilized at 121°C for 3 h. After cooling, sterilized bags were inoculated in the laminar flow cabinet and incubated at 28±2°C without light exposure. Spawn run period completed 12 days. When the mycelium had colonized on the substrate completely, the bags were shifted to cropping room at 30-32ºC, 85-90% relative humidity (RH) with a 10 h light exposure for the formation of fruiting bodies. The mouths of bags were opened and water was sprinkled twice in a day on the bags. Cap formation of *G. lucidum* initiated in 2 to 3 days, after opening the mouths of bags. Fruiting bodies were harvested when the caps become completely red and the white margin disappeared. Total yield (g kg^{-1}) was obtained from three flushes in a harvest period of 60 days. The biological efficiency (BE) percentage [fresh weight of harvested mushrooms/dry matter content of the substrate] × 100 was calculated. Experimental design was a Completely Randomized Block with four replicates. Each

block was placed with 3 plastic bags containing totally 4 kg substrate. The data were analyzed by using the analysis of variance (ANOVA) and group means were compared by Duncan Multiple Range Test (DMR) using the MINITAB program. Correlation analyses were carried out to determine the relationships among yield and biological efficiency (BE) of the substrates.

RESULTS AND DISCUSSION

Sawdust is the most preferred main ingredient used in substrate mixtures for *G. lucidum* cultivation. From the initial studies, it was found that *G. lucidum* took significantly less time for spawn run on Coir pith (100%) as compared to other combinations. Coir pith supplemented with 10 and 20% wheat bran showed quick and complete spawn run (10-12 days) among all the substrate combinations (Figure 1). In case of *D. Sissoo* and its different combinations with wheat bran, incomplete and patchy colonization with thick white rope like mycelial strands was observed. Likewise, Coir Pith it also failed to develop fruiting primordia. Failure of *D. Sissoo* as a substrate medium for *G. lucidum* was also reported in earlier studies (Kapoor, 2011).

Similar results have also been reported stating sawdust as the best substrate for cultivation of *G. lucidum* (Perumal, 2009; Dadwal and Jamaluddin, 2004). Chen (1999) reported Rice bran as an essential ingredient for the cultivation of *G. lucidum* and suggested that addition of WB to the substrate might be taking care of thiamine requirement due to which mycelial growth and reproduction of *G. lucidum* is enhanced (Chen, 1999).

No primordial formation took place, in case of 100% *M. indica*, *P. alba*, Coir Pith and *D. Sissoo*. Combinations of Coir Pith and shesham with wheat bran too failed to form fruiting bodies, thus suggesting them as unsuitable substrate for *G. lucidum*. This might be due to lack of carbohydrate source in case of treatments having 100% sawdust. Reasons for failure of *D. Sissoo* and Coir Pith combinations are yet to be determined.

The results revealed that all the substrates showed significant difference to one another in terms of yield. Among all the different substrates screened for yield performance of *G. lucidum*, *J. mimosifolia* sawdust based combinations were superior in terms of yield (156.0 g kg^{-1}) and biological efficiency (22.33) (Table 1). *J. mimosifolia* sawdust supplemented with 20% WB gave significantly higher yield of 195.0 g kg^{-1} and biological efficiency (27.9%) among all the substrates evaluated and minimum yield (49.4 g kg^{-1}) was obtained with poplar combined with 20%. Among *M. indica* based substrates, *M. indica* sawdust supplemented with 10% WB gave maximum yield with all isolates. Higher yield in local isolates of *G. lucidum* with increase in rate of supplementation was recorded[7]. In the present studies similar trend of increase in yield with supplementation was observed.

Figure 1. Effect of different substrate combinations on *Ganoderma lucidum* (GL18) on different substrate combinations.

Table 1. Yield potential of *Ganoderma lucidum* isolates/strain on various substrate combinations*.

Substrate combinations	Yield	Mean	B.E. (%)	Mean
100% *Mangifera indica* sawdust	88.2[c]		12.6[b]	
90% *Mangifera indica* sawdust + 10% WB**	176.5[b]	138.83[b]	25.2[a]	19.83[b]
80% *Mangifera indica* sawdust + 20% WB	151.8[b]		21.7[a]	
100% *Jacaranda mimosifolia* sawdust	99.0[c]		14.2[b]	
90% *Jacaranda mimosifolia* sawdust + 10% WB	174.0[b]	156.00[a]	24.9[a]	22.33[a]
80% *Jacaranda mimosifolia* sawdust+ 20% WB	195.0[a]		27.9[a]	
100% *Dalbergia sissoo* sawdust	57.8[d]		8.3[c]	
90% *Dalbergia sissoo* sawdust + 10% WB	91.7[c]	66.3[d]	13.1[b]	9.50[c]
80% *Dalbergia sissoo* sawdust + 20% WB	49.4[d]		7.1[c]	

*Means with different letters within a column are significantly different (P<0.05). **WB, Wheat Bran.

These findings are in conformity with some authors who reported hardwood sawdust have been preferred for the commercial production (Chen, 1999) although *Ganoderma* can be cultivated on the sawdust which may originate from different kinds of trees (Wasser, 2005; Olei, 2003; Erkel, 2009).

Kapoor (2011) observed that strain OE53 gave maximum biological efficiency of 17.4% on mango sawdust supplemented with 10% wheat bran (Kapoor, 2011). Rai (2003) reported biological efficiency of *G. lucidum* between 10 to 15% (Rai, 2003). Biological efficiency of 15 to 17% was reported on wheat straw supplemented with 5% rice bran (Mishra and Singh, 2006). The findings of present studies showed significantly higher biological efficiency (27.9%) of isolate GL18 with supplementation of 20% WB on *J. mimosifolia*

sawdust. These results suggest that the yield and BE were effected not only by the sawdust types but also by their combinations. Very little information about the combinations of various kinds of sawdust and wheat bran in the cultivation of *G. lucidum* is available. Most of studies have focused on submerged media in obtaining mycelial biomass. Sawdust of *D. Sissoo* and Coir Pith were found to be most unsuitable material for cultivation of *G. lucidum.*

Conclusions

The effects of various kinds of sawdust and their combination with wheat bran, on the yield of *G. lucidum* were investigated in this study. As described above, yield and biological efficiency of *G. lucidum* varied widely, depending on the kind of sawdust and their combinations. Therefore, it is important to use the proper combination of substrate formulations for the commercial production of *G. lucidum.*

Conflict of Interest

The authors have not declared any conflict of interest.

Abbreviations: BE, Biological efficiency; **BRM,** biological response modifiers; **MEA**, malt extract agar; **WB,** wheat bran; **SD,** saw dust.

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Full Length Research Paper

Sample scaling in soil compaction assessment experiments with motorized penetrometer

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The objective was to estimate the sample size (number of points by experimental plot) to estimate soil resistance to penetration in different depth ranges and for different animal stocking density rates. The data was obtained from a factorial experiment in a randomized block design, three replicates, consisting of two grazing intensity levels (canopy heights: 10 and 20 cm) and two levels of nitrogen fertilization applied on the coverage (0 and 200 kg N ha^{-1}) in the form of urea. The penetration resistance (PR) of the soil was achieved in 20 points randomly determined inside each experimental unit (paddock). A "Motorized soil penetrometer" was used (DLG, Model PNT-2000-M). The PR average values were determined for the depth range of the soil (characters): 00-40, 00-10, 10-20, 20-30 and 30-40 cm. For the same estimation error of the soil's penetration resistance, the sample size (number of points) depends on the depth range of soil and animal stocking density. In experiments with varying animal stocking density rate on pasture in winter, 15 points per experimental unit are sufficient to estimate the average with an estimation error of 15% of the average soil penetration resistance.

Key words: Sample size, grazing pressure, crop-livestock integration, soil depth.

INTRODUCTION

The feasibility of the integrated crop-livestock system depends on the management adopted in the grassland and grazing pressure during the winter, as this affects the performance of cash crop plants in summer, whether for the production of grain or forage. However, the use of different grazing pressures can result in different levels of soil compaction, due to animal treading, even more that the grazing pressure changes animal movement patterns (Baggio et al., 2009), leading to different soil compaction patterns. Differences in the degree of compaction may

determine mechanical difficulties at sowing and change the density and regularity of plant density, with consequences for the crop yield.

The correct measure of soil compaction in experiments, by using the equipment known as "Penetrometer", is essential for improving experimental accuracy and labor efficiency. The penetrometer allows the measurement of one of the most important physical properties of the soil, the penetration resistance (PR). This property is related to various soil properties, which are indicators of the

degree of compaction (Tavares Filho and Ribon, 2008).

The importance of the representativeness of measurements (sample size) is also known in the experimental units to reduce experimental error and, consequently, increase the accuracy of the research results (Cargnelutti Filho et al., 2011; Storck et al., 2012; Benin et al., 2013). The diagnosis of soil compaction in large areas requires time and it is labor-intensive, especially when working with precision agriculture, in which there is the need to sample many points (Molin et al., 2012). Tavares Filho and Ribon (2008) point out that studies on the number of sample points are scarce, since, in general, they are set out aimed at better value for money, which do not necessarily present statistically reliable results. PR measurements are being conducted with various sampling plans, with no differences in the number of points, when the distribution is systematic (mesh) or when it is at random points (Tavares Filho and Ribon, 2008). Also, the sample size can vary with the management system of the pasture and the sampling depth range.

In the absence of information about the size of the experiments, the number of sampled points per experimental unit has been variable. There are cases with less than 10 points (Lima et al., 2013; Freitas et al., 2012; Silveira et al., 2010; Moraes et al., 2012) and cases with 10 to 20 (Ralisch et al., 2008; Tavares Filho and Ribon, 2008) per study unit, size not specified. Other studies used sample sizes equal to 16 (Coelho et al., 2012), 570-1333 (Mome Filho et al., 2014), 1,111 (Roque et al., 2008), 7,100 (Iaia et al., 2006) and 11,100 (Molin et al., 2012) points ha^{-1}.

Possibly, the number of sampling points is related to the operation of the equipment, gathering of data from other variables at the same points and the availability of human resources. In order to determine the number of evaluation points (sample size) from PR data of 40 points sampled in three crops (Molin et al., 2012), they observed that from 15 points (replicates) the trend is for the standard error to present very similar values in all three fields, between 5 and 15% of the average, though without significant decrease in their values by increasing the number of points. Tavares Filho and Ribon (2008) concluded that there is a variation in the sample size in relation to the management system and sampling depth. Also, the effect of grazing management on the sample size to estimate the PR is not known. The objective was to estimate the sample size (number of points by experimental plot) to estimate soil resistance to penetration in different depth ranges and for different animal stocking densities.

MATERIALS AND METHODS

The "crop-livestock integration" experiment was conducted in a property located in the city of Abelardo Luz, Santa Catarina State, Brazil (26º 31' 34" S; 52 º 15' 36" W; altitude 851 m). According to the Brazilian system of soil classification (Santos et al., 2013), the

soil of the area is classified as "Latossolo Bruno distrófico" (Dusky latosol, dystrophic, typical), very clayey texture (69.5% clay, 26.8% silt and 3.7% sand) with prominent horizon A (between 0 and 39 cm).

The factorial experiment in a randomized block design, three replicates, consists of two grazing intensity levels (grazing pressure) through the grazing method with continuous stocking varying stocking density (Mott and Lucas, 1952), seeking to maintain two sward canopy heights of black oat (*Avena strigosa*, cv. BRS 139.) + annual ryegrass (*Lolium multiflorum* cv. Barjumbo), 20 and 30 cm, and two levels of nitrogen fertilization applied once, on the top: 0 and 200 kg N ha^{-1}, in the form of urea. These four managements result in various numbers of animals per area unit, which depends on the forage availability. The total area of the 12 experimental units of the experiment is equal to 16 ha, approximately 1.2 ha per working experimental unit. The forage (oat + ryegrass) was sowed on 04/03/2014, and the grazing started on 20 May, 2014. On the 10 October, 2014, the cattle were removed from the experimental area aiming at preparing for soybean cultivation. On 13 November 2014 the data were collected for penetration resistance of the soil and soil moisture. Soil moisture was determined, at one point per experimental unit at depths of 0-5, 5-10, 10-20 and 20-40 cm. The results were submitted to analysis of variance per depth.

The penetration resistance of the soil (PR) was performed in 20 randomized points in each paddock. For this operation we used a "digital motorized soil penetrometer" (DLG, Model PNT-2000-M), recording the values of PR every 10 mm deep (between the surface and 400 mm), using the cone type 2 (129 mm[2]). The measurement unit was the soil resistance to the penetration of the cone, expressed in MPa (Mega Pascal). The PR average values were determined for the depth range of the soil: 00-40, 00-10, 10-20, 20-30 and 30-40 cm, thus representing five evaluated layers.

For each layer, variance analysis was conducted according to a randomized block design with sampling in the experimental unit. Assumptions were tested regarding the management effect and experimental error (variance among experimental units) according to Barbin (1998). Were also tested the assumptions of error normality and homogeneity of variances between managements (Barbetta et al., 2004). For this analysis, we used the Genes software (Cruz, 2013).

The PR mean (m) was estimated for each management and layer (20 points x three blocks), and the within experimental unit variance (s^2 = mean of the 20-point variances in three block). We calculated the sample size (η) for the confidence interval with half-width (HW) equal to 5, 10, 15 and 20% of the mean (m) estimate with a confidence level (1-α) of 95% through the expression $\eta = t_{\alpha/2}^2 s^2 / (HW)^2$ (Barbetta et al., 2004), in which $t_{\alpha/2}$ is the critical value of the *Student's* t distribution such that $P(t > t_{\alpha/2}) = \alpha/2$ with (n-1) degrees of freedom with α = 5% error probability, and s^2 is the variance estimate. Later, η as fixed as the total points (N = 20) used to calculate the half-width of the confidence interval (HW20, 1-α = 0.95) as a percentage of the mean (m) estimate for each management and depth range through the expression $HW20 = 100 \, t_{\alpha/2} s / m\sqrt{\eta}$ (Barbetta et al., 2004), where *s* is the sample standard deviation estimate. For the calculations, we used the resources of Excel® spreadsheet.

RESULTS AND DISCUSSION

Soil compaction, estimated by the penetration resistance of the soil (PR) was significantly ($p < 0.05$) affected by management only in the 10-20 cm layer (Table 1).

Table 1. Analysis of variance with degrees of freedom (DF), sources of variation (SV) for the resistance of the soil to penetration (MPa), by depth range; average, selective accuracy (SA), p-value of the Kolmogorov-Smirnov test (α-KS) and p-value of the Bartllet test (α-Bartllet) between managements.

SV	DF	Mean square for the layers				
		00-40	00-10	10-20	20-30	30-40
Block	2	0.6603	0.7722	0.8070	3.1515	5.1815
Management	3	0.8457^{ns}	3.2678^{ns}	6.8169^{*}	1.1553^{ns}	2.2729^{ns}
Among	6	1.9116^{*}	1.6394^{*}	0.6110^{ns}	3.0201^{*}	8.9396^{*}
Within	228	0.2158	0.5455	0.3497	0.2854	0.3218
Average	-	2.7264	3.4376	3.0593	2.2912	2.1178
SA	-	-	0.706	0.954	-	-
α-KS	-	0.699	0.773	0.813	0.668	0.711
α-Bartllet	-	0.045	0.065	0.003	0.008	0.004

* Significant effect by F test (α <0.05); ^{ns} = not significant.

Therefore, after 172 days of grazing, compaction in the 0-20 cm layer was affected by managements of animal stocking density and nitrogen. In this same layer (10-20 cm) the heterogeneity of variance among replicates was not significant, and, according to this result, the increase in the number of replications is less important than the increase of sample size to reduce the average estimated variance of the managements according to Barbin (1998). The reverse occurs with the remaining layers where the management effects were not significant.

The extent of the experimental precision, selective accuracy (SA = $(1-1/Fc)^{0.5}$), is classified as very high (Resende and Duarte, 2007; Benin et al., 2013) in the 10-20 cm layer; and high in the 0-10 cm, although with no management effect. In the cases where the F-value for management is smaller than one, there is no estimate of SA and a sampling plan that uses the same number of points of management should provide a larger sample size in detriment of the number of repetitions for the comparison of management with greater accuracy (Barbin, 1998).

For the other layers and in the average (00-40 cm) there is no effect of the management on the PR. The cause of no significance in the management of these bands may be due to the high value of the variation among the experimental plot (experimental error) that was significant. In this case, the experimental error is equivalent to the interaction "Management x Block" estimable for cases of sampling in the experimental units (Barbin 1998). If there is an interaction, differences in management practices within a block do not have the same order in relation to the other blocks, overcoming the main effect of management.

The overall average of PR varies between 2.12 and 3.44 MPa for the layers. Studies report that the value of 2.0 MPa has been accepted as the critical threshold of soil resistance to penetration (Taylor et al., 1966; Nesmith, 1987) to prevent crop yields. However, for Vepraskas and Miner (1986), values of 2.8 to 3.2 MPa

slow elongation of roots and at 4.0 MPa there is no growth of roots. Another study concluded that PR values greater than 3.5 MPa did not restrict root development of corn, but influences its morphology (Tavares Filho et al., 2001). Thus, the degree of soil compaction observed in this study is close to the limit tolerated for a good plant growth.

Soil moisture (g g^{-1}) at the time of determination of the PR was 21.7% in the 0-5 cm layer; 24.5% in the 5-10 cm layer; 26.4% in the 10-20 cm layer; and 29.1% in the 20-40 cm layer. No significant effect of the managements on soil moisture was seen in the evaluated layers ranges. With this, the differences in PR values for the various managements are not related to soil moisture. However, in other studies, the magnitude of the PR is also related to soil management (Freitas et al., 2012; Ralisch et al., 2008; Girardello et al., 2014).

The assumptions of normality of the errors (Table 1) are not restricted to the hypothesis testing for the evaluated depth ranges. Thus, it is possible to use the t-distribution to estimate sample size. However, due to the heterogeneity of the variances among managements, it is not recommended to use a single sample size for the different managements. Heterogeneous (Cargnelutti Filho et al., 2010) and homogeneous (Krause et al., 2013) variances between treatments were also observed in other studies and were attributed to the effects of the treatments.

Considering that, to keep highest forage production in management M1 and M2 in relation to the managements M3 and M4 (Table 2), it was necessary to lower the animal stocking density rate (less grazing pressure) and when nitrogen is used in the pasture, management M1 and M3 the plants perform better. In these cases, there is the need to increase animal stocking density to maintain the same sward canopy height of the plant. Thus, there are two extremes of managements that influence soil PR, the M1 with lower rate of animal stocking density and M4 with the highest rate. Similarly, it should be noted that

Table 2. Sample size (number of points) for estimating the average soil resistance to penetration (PR, MPa) in portions under different managements, for estimation errors equal to half-width (HW = 5, 10, 15 and 20%) of the PR average estimation (α = 0.05) and half-width of confidence interval based on the number of measured points (HW20, n = 20) in percentage, average PR and average variance.

HW (%)	Soil depth ranges (cm)				
	0-10	10-20	20-30	30-40	0-40
M1 = Managements without N application and high sward canopy height					
5	47	57	104	119	40
10	12	14	26	30	10
15	5	6	12	13	4
20	3	4	7	7	3
HW20	7.7	8.5	11.4	12.2	7.1
Average PR	2.664	3.700	2.849	2.177	1.931
Average variance	0.178	0.400	0.290	0.307	0.277
M2 = Management with N application and high sward canopy height					
5	89	45	98	109	51
10	22	11	25	27	13
15	10	5	11	12	6
20	6	3	6	7	3
HW20	10.6	7.5	11.1	11.7	8.0
Average PR	2.641	3.478	2.890	2.178	2.020
Average variance	0.222	0.674	0.234	0.291	0.277
M3 = Management without N application and low sward canopy height.					
5	58	45	65	99	35
10	14	11	16	25	9
15	6	5	7	11	4
20	4	3	4	6	2
HW20	8.5	7.5	9.0	11.1	6.6
Average PR	2.699	3.132	2.937	2.349	2.380
Average variance	0.159	0.355	0.244	0.225	0.349
M4 = Management with N application and low sward canopy height					
5	102	80	84	134	58
10	26	20	21	34	15
15	11	9	9	15	6
20	6	5	5	8	4
HW20	11.3	10.0	10.3	13.0	8.5
Average PR	2.901	3.440	3.562	2.460	2.141
Average variance	0.304	0.754	0.630	0.318	0.384

when the animal stocking density rate is higher, and therefore the forage mass is higher, ruminants grazing walk less, although the distance between feeding stations increases, the time spent per feeding station increases and the bites rate decreases (Baggio et al., 2009).

Considering the foregoing, it is fully understandable that in this study, the management with the most animal stocking density (M4 management) shows greater heterogeneity of the area. This is due, possibly to the routes of the animals journeys and choice (random) of the 20 points (129 mm^2 point^{-1}) of the PR's sampling, which may or may not match the area of the animals footprints (±100 cm^2). Other managements with fewer circulating animals are less likely to coincide a measurement point of the PR with the animal trod; this fact increases the heterogeneity among points.

A variation is observed in sample size between soil depth ranges and between the managements and for each estimation error (Table 2). Considering there was heterogeneity of variances among managements (Table

1), sample sizes should also be different. In this case, to maintain the level of significance of all managements of the experiment, one should adopt for the larger sample size between the management systems for the same magnitude of the estimation error. Considering also that the PR evaluation is unusual in one or other range of depth, one should also choose the sample size on the higher value range. In this study, for an estimation error equal 10% (HW, half-width of confidence interval = 10% from the average), the sample size would be equal to 34 points, corresponding to the management "With application of N and low sward canopy height"; in 30-40 cm layer. To match the availability of financial and human resources, It can be adjusted the magnitude of the estimation error for the feasible sample size (Table 2). Thus, for an estimation error of HW = 15%, the sample size is 15 points and eight points for an estimation error of HW = 20%. Considering, in this study, that we used 20 points per experimental unit, the estimation error (HW20) for this sample size has a maximum value among managements and depths equal to 13% of the average.

It was found, in another study, that significant decreases do not occur in the standard error values by increasing the number of points beyond 15, ranging between five and 15% among penetrometers, within each sampled area (Molin et al., 2012). A variation in sample size in relation to the management system and the soil layers was also reported. In addition, the sample size for a 10% estimation error of the mean is n = 15 points for no-till system and perennial crops soils; and n = 20 points for conventional till (Tavares Filho and Ribon, 2008). For chemical analyzes of soil samples, the collection of at least eight single soil samples would be enough to form a representative composite sample for evaluation of average soil fertility in a seemingly homogeneous sampling unit, but the reliability or accuracy of the estimate the medium fertility will be higher the larger the number of single samples collected to form a composite sample (Santos et al., 2009). In this study, this would be equivalent to increasing the number of PR measurement points per experimental unit.

Conclusions

For the same estimation error of the soil's penetration resistance, the sample size (number of points) depends on the depth range of soil and animal stocking density. In experiments with varying animal stocking density on cool-season grasses pasture, 15 points per experimental unit are sufficient to estimate the average with an estimation error of 15% of the average soil penetration resistance.

Conflict of Interest

The author(s) have not declared any conflict of interest.

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