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*Full Length Research Paper*

# Agronomic performance evaluation of sugarcane varieties under Finchaa Sugar Estate agro-ecological conditions

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**Field experiment was conducted at Finchaa Sugar Estate, to evaluate and select sugarcane varieties with better agronomic performances under Finchaa agro-ecological condition. Eleven sugarcane varieties are namely, :B58 230, N53 216, N52 219, M202/46, CP47/193, DB386/60, B59 250, COK 30, B60 163, CO 1148, BO 60349 and NCo334 (Check variety) were evaluated in completely randomized block design with three replications. Result indicated that sugarcane variety N53 216 produced significantly highest sugar yield of 1.7 and 1.8 $tha^{-1}$ $month^{-1}$ in Luvisol and Vertisol respectively, and it gave 25 to 28% yield advantage over the check variety NCo 334. The next best variety in sugar yield was BO 60349 (1.4 $tha^{-1}month^{-1}$) for Luvisol and B60 163 (1.5 $tha^{-1}month^{-1}$) for Vertisol and it was on par with B58 230 (1.4 $tha^{-1}month^{-1}$ for both Luvisol and Vertisol). Furthermore, except for sugarcane varieties B59 250 in both soil types and varieties N52 219, M202/46 and COK 30 in luvisol the rest were not significantly different from the check variety NCo 334. Thus, the sugarcane varieties N53 216, B58 230, BO 60349, B60 163, CP47/193, DB386/60, and CO 1148 in both soil; whereas N52 219, M202/46 and COK 30 only in luvisol selected to be verified further in large commercial fields at Finchaa Sugar Estate.**

**Key words:** Luvisol, plant cane, ratoon, sugarcane, variety, vertisol.

## INTRODUCTION

Sugarcane (*Saccharum* spp.) is an important economic crop in the tropics and sub-tropics due to its high sucrose content and bioenergy potential. Sugarcane in Ethiopia so far the industry does not have its own breeding program it has always been dependent on importing sugarcane varieties and locally evaluating their performance on yield and diseases.

According to Sundara (2000), in order to enhance productivity and profitability of commercial scale sugarcane cultivation, adoption of high yielding varieties and improved production packages are highly demanding. There are number of reasons for lower cane yield and one of those is the planting of low yielding varieties. Therefore, it is need of the time to introduce

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new high yielding varieties with good ratoon ability in the country (Chattha and Ehsanullah, 2003). Variety plays a key role in both increasing and decreasing per unit area sugar yield, while use of unapproved, inferior quality cane varieties affect sugarcane production negatively as situation prevails today (Mian, 2006). The solution of low cane yield and sugar recovery problem lies in the planting of improved cane varieties (Chattha et al., 2006). Varieties differ not only in their yield of cane but also in their juice quality. They also differ in the length of time required to reach maturity. There are also very marked responses to the environment, and even different ecological zones within a country. It is for this reason that, if the best selection is to be made, the final stages in a varietal selection program must incorporate trials in a country that must be representative of all the main ecological zones (James, 2004). Important considerations in choosing an appropriate variety are: cane yield, juice quality, age group, suitability to the growing condition (that is, soil type, irrigation level, season etc.), ratooning potential, and resistance to disease and pest and adverse growing condition (Sundara, 2000).

Efforts are being made to increase cane production by introducing high yielding varieties and adoption of improved crop production techniques (Gill, 1999). Success of variety depends upon its adaptability to agro-climatic conditions of the area. Selection of a proper variety to be sown in a particular agro-ecological zone is a primary requisite to explore its yield and sugar recovery potential. Ratoons are important for overall profitability of sugarcane cultivation as they save about 30% in the operational cost, mainly that of seed and reduced expenses for soil management (Sundara et al., 1992). The inherent potential of a variety to give better yields in plant and ratoon crops is of paramount importance for sustaining high productivity. Acceptance of a variety by the farmers now depends very much on its ratooning potential. Thus, sugarcane varieties, which show good performance in plant and ratoon crops, should be promoted for commercial cultivation. In Ethiopia, even if the country has a fertile soil and favorable environmental condition for sugarcane production its average cane yield is limited to about 104 ton/ha/year (Sugar Corporation, 2011) this could be attributed by many factors, of which lack of improved varieties play central role. For this purpose, the variety improvement strategy should guarantee proper substitution of declining or poor performing commercial varieties from its sugarcane germplasm pool following proper field evaluation. Therefore, the government has developed a strategy to import divers improved sugarcane varieties from around similar ecological locations of the glob to secure the upcoming huge development and expansion in sugar industry.

Accordingly, 10 promising sugarcane varieties from the introduced materials, were promoted and tested under two soil types of Finchaa agro-ecological condition to identify elite candidate for pre-commercial release. Therefore, the present study was conducted to identify better performer variety/varieties in sugar yield under Finchaa agro-ecological condition.

## MATERIALS AND METHODS

### Description of the study area

The study was conducted at Fincha'a Sugar Estate during 2003/4 to 2008/9 cropping season. The area is found 330 km west of Addis Ababa, and is located at 9° 31' to 10° N latitudes and 37° 15' to 37° 30' E longitude with an elevation between 1350 and 1650 m a.s.l. The area characterized by average annual rainfall of 1280 mm with a mean minimum and maximum temperature of 14.5°C and 30.6°C, respectively. Moisture demand of the crop is supplemented by sprinkler irrigation.

### Experimental design and treatments

The study comprised, eleven test varieties that were promoted from disease evaluation trial and a check variety with better adaptation were considered: B58 230, N53 216, N52 219, M202 46, CP47 193, DB386 60, B59 250, COK 30, B60 163, CO 1148, BO 60349 and NCO334 (Check variety).

All the varieties considered in this study were newly introduction to Finchaa Sugar Estate and were selected based on their performance on variety adaptability trials conducted at Wonji - Shoa and/or Metahara Sugar Estates (Aregaw, 1997, 2000). The evaluation was made based on plant cane and two ratoon crops on the two major soil types of Finchaa (Vertisol and Luvisol). The experiment was laid out in a Randomized Complete Block Design with three replications. Each experimental plot had a size of 29.0 $m^2$ (four furrows of 5 m length and 1.45 m space between furrows). For data collection and observation, only the two middle rows were considered. The distance between plots and within replication was 1.5 and 2.9 m, respectively.

### Parameters collected and data analysis

During the course of the experiment sprout percent, tiller count, number of millable canes and growth (height) measurement were taken. Moreover cane yield, cane thickness (girth), number of internodes, percent sucrose and estimated sugar yield were measured at harvest by taking 10 random samples from each plot. Except for the varieties used as treatments in this trial other inputs and field operations on the site were made following conventional practices of the Estate.

Finally, data were subjected to General Linear Models Procedure (GLM) using SAS software statistical package (SAS, 2002) following a procedure appropriate to the design of the experiment (Gomez and Gomez, 1984). The treatment means that were significantly different at 5% levels of significance were separated using the Duncan Multiple Rang Test (DMRT).

## RESULTS AND DISCUSSION

### Sprouting, tillers and millable canes

Combined analysis of the data on plant cane, 1$^{st}$ and 2$^{nd}$

**Table 1.** Pooled mean performance for 9 traits of 12 sugarcane varieties grown at 2 soil types - plant cane.

| Varieties (V) | Sprouting (%) | Tiller no. ('000' ha$^{-1}$) | No. of internode | Cane height (cm) | Cane thickness (mm) | Millable cane ('000' ha$^{-1}$) | Cane yield (t ha$^{-1}$ month$^{-1}$) | Sucrose %cane | Sugar yield (t ha$^{-1}$ mon$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| B58 230 | 60.3$^{c}$ | 246.27$^{ef}$ | 17.6$^{def}$ | 214.5$^{fg}$ | 26.6$^{b}$ | 130.518$^{ef}$ | 11.26$^{a}$ | 11.75$^{cd}$ | 1.384$^{ab}$ |
| N53 216 | 82.5$^{a}$ | 289.32$^{bcd}$ | 18.5$^{cd}$ | 224.8$^{ef}$ | 24.7$^{c}$ | 148.967$^{bc}$ | 10.66$^{a}$ | 13.79$^{a}$ | 1.483$^{a}$ |
| N52 219 | 33.3$^{d}$ | 158.35$^{h}$ | 20.4$^{a}$ | 234.5$^{de}$ | 24.8$^{c}$ | 103.739$^{h}$ | 10.08$^{ab}$ | 12.46$^{bc}$ | 1.332$^{ab}$ |
| M202 46 | 67.0$^{bc}$ | 332.53$^{a}$ | 16.6$^{fg}$ | 206.2$^{g}$ | 30.8$^{a}$ | 92.531$^{h}$ | 9.45$^{abc}$ | 10.09$^{e}$ | 1.195$^{bc}$ |
| CP47 193 | 73.5$^{ab}$ | 335.97$^{a}$ | 19.9$^{ab}$ | 250.9$^{bc}$ | 21.0$^{e}$ | 167.185$^{a}$ | 9.60$^{abc}$ | 12.22$^{bcd}$ | 1.261$^{ab}$ |
| DB386 60 | 73.5$^{ab}$ | 319.48$^{ab}$ | 17.1$^{efg}$ | 228.8$^{def}$ | 23.2$^{d}$ | 134.080$^{def}$ | 7.94$^{c}$ | 12.63$^{b}$ | 0.983$^{cd}$ |
| B59 250 | 43.0$^{d}$ | 258.52$^{def}$ | 16.4$^{g}$ | 240.7$^{cd}$ | 19.1$^{f}$ | 158.506$^{ab}$ | 8.81$^{bc}$ | 7.13$^{f}$ | 0.793$^{d}$ |
| COK 30 | 65.2$^{bc}$ | 302.87$^{abc}$ | 17.3$^{efg}$ | 270.8$^{a}$ | 21.6$^{e}$ | 143.852$^{dc}$ | 11.06$^{a}$ | 11.60$^{d}$ | 1.384$^{ab}$ |
| B60 163 | 73.7$^{ab}$ | 190.63$^{gh}$ | 17.8$^{de}$ | 261.7$^{ab}$ | 27.3$^{b}$ | 115.575$^{g}$ | 8.32$^{bc}$ | 11.55$^{d}$ | 0.936$^{d}$ |
| CO 1148 | 69.5$^{bc}$ | 335.37$^{a}$ | 19.1$^{bc}$ | 273.0$^{a}$ | 23.5$^{d}$ | 134.600$^{de}$ | 10.88$^{a}$ | 10.53$^{e}$ | 1.243$^{ab}$ |
| BO 60349 | 60.7$^{c}$ | 268.10$^{cde}$ | 18.0$^{de}$ | 228.6$^{def}$ | 27.0$^{b}$ | 121.954$^{fg}$ | 9.82$^{ab}$ | 11.78$^{cd}$ | 1.244$^{ab}$ |
| NCo 334 | 42.0$^{d}$ | 223.68$^{fg}$ | 19.5$^{abc}$ | 219.5$^{efg}$ | 24.0$^{cd}$ | 141.438$^{cde}$ | 10.74$^{a}$ | 11.60$^{d}$ | 1.414$^{ab}$ |
| **LSD (0.5%)** | **10.2** | **34.13** | **1.02** | **13.75** | **0.86** | **11.63** | **1.59** | **0.72** | **0.22** |
| **Soils (S** | | | | | | | | | |
| Luvisol | 65.2$^{a}$ | 276.30$^{a}$ | 18.41$^{a}$ | 241.3$^{a}$ | 24.7$^{a}$ | 126.880$^{b}$ | 9.89$^{a}$ | 11.47$^{a}$ | 1.221$^{a}$ |
| Vertisol | 58.8$^{b}$ | 267.203$^{a}$ | 17.93$^{b}$ | 234.4$^{b}$ | 24.3$^{b}$ | 138.611$^{a}$ | 8.67$^{b}$ | 11.38$^{a}$ | 1.088$^{b}$ |
| **LSD (0.5%)** | **3.9** | **13,933** | **0.42** | **5.6** | **0.35** | **4.75** | **0.58** | **0.29** | **0.078** |
| **V x S** | **NS** | **NS** | **NS** | **NS** | **NS** | **NS** | **NS** | **NS** | **NS** |
| **CV (%)** | **14.14** | **4.84** | **4.84** | **4.97** | **3.03** | **7.54** | **13.84** | **5.40** | **15.33** |

Means with the same letter are not significantly different at P≤.05.

ratoon; except tillers of 1$^{st}$ ratoon revealed no interaction between varieties and soil types on percent sprouting, number of tillers and number of millable canes (Appendix Tables 1). Whereas, sugarcane varieties were significantly different from each other on percent sprouting, number of tillers and number of millable canes in all the three cuttings (Tables 1, 2 and 4).

Percent sprouting for twelve sugarcane varieties and the difference among them are presented in Table 1. Remarkably higher percent of sprouting for all varieties were obtained from Luvisol than the one obtained from Vertisol (Table 1), and this result agrees with previous result reported by Worku and Chinawong (2006). Greater percent of sprouting was obtained for varieties N53 216 (82.5%), B60 163(73.7%), CP47 193 (73.5%), DB386 60 (73.5%) and CO 1148 (69.5%).

On plant cane significantly higher number of tillers per hectare was recorded from variety CP47 193 (335. 977 × $10^3$), CO1148 (335.345 × $10^3$) and DB386 60 (319.483 × $10^3$). But there was no statistical difference between the two soil types (Table 1). In first ratoon cane of Luvisol significantly more number of tillers was recorded from variety CO1148 (400 X $10^3$), B59 250 (377.24 X $10^3$), M202 46 (373.908 × $10^3$), and DB386 60 (366.092 × $10^3$). On Vertisol, Variety N53 216 (352.299 × 103) produced relatively higher number of tillers followed by M202 46 (346.552 × $10^3$), COK 30 (333.334 × $10^3$), and B59 250 (320.46 × $10^3$) (Table 2). Again N53 216 (345.575 ×$10^3$) was the 1$^{st}$ in number of tillers of second ratoon, followed by B59 250 (340.345 × $10^3$).

Statistically, significantly more number of tillers per hectare was recorded in Luvisol (325.489 × $10^3$) than Vertisol (Table 4). In all cuttings variety N52 219 show inferior number of tillers per

**Table 2.** Pooled mean performance for 8 traits of 12 sugarcane varieties grown at 2 soil types - 1st Ratoon.

| Varieties | Tiller no. ('000' ha$^{-1}$) | No. of inter-node | Cane height (cm) | Cane thickness (mm) | Millable cane ('000' ha$^{-1}$) | Cane yield (tha$^{-1}$ month$^{-1}$) | Sucrose % cane | Sugar yield (tha$^{-1}$ month$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| B58 230 | 314.48$^{cd}$ | 25.0$^{bcd}$ | 253.7$^{e}$ | 25.5$^{b}$ | 148.80$^{cde}$ | 12.25$^{ab}$ | 13.16$^{cd}$ | 1.61$^{b}$ |
| N53 216 | 363.39$^{a}$ | 25.0$^{bcd}$ | 271.6$^{de}$ | 23.5$^{c}$ | 173.16$^{ab}$ | 13.59$^{a}$ | 14.06$^{a}$ | 1.91$^{a}$ |
| N52 219 | 249.37$^{e}$ | 26.7$^{abc}$ | 290.7$^{bcd}$ | 23.0$^{c}$ | 133.28$^{efg}$ | 10.71$^{bc}$ | 12.65$^{cd}$ | 1.35$^{cd}$ |
| M202 46 | 360.23$^{a}$ | 24.8$^{bcd}$ | 257.3$^{e}$ | 29.2$^{a}$ | 114.19$^{g}$ | 10.08$^{bc}$ | 12.12$^{d}$ | 1.33$^{d}$ |
| CP47 193 | 318.97$^{bcd}$ | 27.5$^{a}$ | 300.2$^{abc}$ | 20.2$^{d}$ | 192.99$^{a}$ | 11.83$^{bc}$ | 12.67$^{cd}$ | 1.50$^{bcd}$ |
| DB386 60 | 330.75$^{abc}$ | 24.3$^{cd}$ | 274.6$^{de}$ | 22.7$^{c}$ | 141.44$^{def}$ | 10.63$^{bc}$ | 13.52$^{abc}$ | 1.44$^{cd}$ |
| B59 250 | 348.85$^{ab}$ | 24.3$^{cd}$ | 321.4$^{a}$ | 18.5$^{e}$ | 191.55$^{a}$ | 9.05$^{d}$ | 9.35$^{e}$ | 0.86$^{e}$ |
| COK 30 | 343.79$^{abc}$ | 23.3$^{d}$ | 317.6$^{a}$ | 19.7$^{de}$ | 158.16$^{bcd}$ | 10.38$^{cd}$ | 13.30$^{abc}$ | 1.38$^{bcd}$ |
| B60 163 | 227.24$^{e}$ | 25.0$^{bcd}$ | 285.6$^{cd}$ | 25.2$^{b}$ | 128.85$^{efg}$ | 11.79$^{bc}$ | 13.58$^{abc}$ | 1.60$^{bc}$ |
| CO 1148 | 351.72$^{a}$ | 25.2$^{bcd}$ | 313.2$^{ab}$ | 22.8$^{c}$ | 133.96$^{efg}$ | 11.67$^{bc}$ | 12.83$^{bcd}$ | 1.50$^{bcd}$ |
| BO 60349 | 254.77$^{e}$ | 24.8$^{bcd}$ | 260.2$^{e}$ | 25.0$^{b}$ | 121.54$^{fg}$ | 10.39$^{cd}$ | 13.84$^{ab}$ | 1.44$^{bcd}$ |
| NCo 334 | 298.62$^{d}$ | 26.8$^{ab}$ | 257.0$^{e}$ | 22.2$^{c}$ | 168.58$^{bc}$ | 12.01$^{bc}$ | 13.13$^{abcd}$ | 1.58$^{bc}$ |
| **LSD (0.5%)** | 29,192 | 2.04 | 23.2 | 1.33 | 21.32 | 1.42 | 0.95 | 0.21 |
| **Soils (S)** | | | | | | | | |
| Luvisol | 327.59$^{a}$ | 30.36$^{a}$ | 316.1$^{a}$ | 23.97$^{a}$ | 142.12$^{b}$ | 10.82$^{b}$ | 12.75$^{a}$ | 1.38$^{b}$ |
| Vertisol | 299.44$^{b}$ | 20.11$^{b}$ | 251.1$^{b}$ | 22.25$^{b}$ | 158.97$^{a}$ | 11.74$^{a}$ | 12.93$^{a}$ | 1.54$^{a}$ |
| **LSD (0.5%)** | 11.92 | 0.83 | 9.5 | 0.54 | 8.70 | 0.58 | 0.39 | 0.09 |
| **V x S** | * | **NS** | **NS** | **NS** | **NS** | **NS** | *** | **NS** |
| **CV (%)** | 8.01 | 6.95 | 7.04 | 4.96 | 12.19 | 10.87 | 6.36 | 12.45 |

Means with the same letter are not significantly different at P≤0.05.

hectare (Tables 1, 2 and 4), except in Luvisol of 1st ratoon, that is, B60 163 (Table 2). Furthermore both plant cane and first ratoon of all the test varieties were superior in number of tillers than the check variety NCO 334.

The survival of the tillers and reaching the status of millable cane was significantly higher in CP47 193 (167.185 × $10^3$, 192.99 × $10^3$ and 163.74 × $10^3$ in PC, 1St ratoon and 2nd ratoon, respectively) and B59250 (158.506 × $10^3$, 191.55 × $10^3$, and 187.19 × $10^3$ in PC, 1St ratoon and 2nd ratoon, respectively) while it was significantly least in M202 46 in all the three cuttings. The increment in numbers at the early stage of growth and the reduction of stalk population during the growth of sugarcane is a characteristic of several gramineous.

This reduction of stalk population (mortality of cane) could be attributed to the factors which induce competition for light, moisture and nutrient; and the survival of the tillers after the competition is a character of a variety. Thus, in the present finding the variation in survival and mortality rate could be probably attributed to the differences in the genetic makeup of the varieties (Worku and Chinawong, 2006).

**Number of inter- node, cane height, and cane thickness (girth)**

Analysis of variance of number of inter-nodes, cane height, and cane thickness resulted in significant main effects of varieties and soil types on all the traits for all the three cuttings (plant cane, first and second ratoon). However, their interaction was not significant on any of the traits except on the cane height of the second ratoon (Appendix Table 1).

In number of inter- node variety N52219 and CP47 193 were not significantly different from the

**Table 3.** The effects of variety and soil type on tiller number and sucrose content - 1st ratoon.

| Varieties | Tiller no. | | Sucrose % Cane | |
|---|---|---|---|---|
| | Luvisol | Vertisol | Luvisol | Vertisol |
| B58 230 | 323.91$^{cd}$ | 305.06$^{ab}$ | 13.49$^{ab}$ | 12.83$^{cd}$ |
| N53 216 | 374.48$^{ab}$ | 352.30$^{a}$ | 13.37$^{ab}$ | 14.76$^{a}$ |
| N52 219 | 266.67$^{e}$f | 232.07$^{c}$ | 12.14$^{bc}$ | 13.15$^{bc}$ |
| M202 46 | 373.91$^{ab}$ | 346.55$^{a}$ | 12.54$^{abc}$ | 11.70$^{d}$ |
| CP47 193 | 327.701$^{cd}$ | 310.23$^{ab}$ | 11.02$^{cd}$ | 14.31$^{ab}$ |
| DB386 60 | 366.09$^{ab}$ | 295.40$^{ab}$ | 13.30$^{ab}$ | 13.74$^{abc}$ |
| B59 250 | 377.24$^{ab}$ | 320.46$^{a}$ | 10.30$^{d}$ | 8.40$^{e}$ |
| COK 30 | 354.25$^{bc}$ | 333.33$^{a}$ | 13.21$^{ab}$ | 13.39$^{abc}$ |
| B60 163 | 220.58g | 233.91$^{c}$ | 13.82$^{ab}$ | 13.34$^{bc}$ |
| CO 1148 | 400.00$^{a}$ | 303.45$^{ab}$ | 12.72$^{ab}$ | 12.94$^{bcd}$ |
| BO 60349 | 252.87fg | 256.67$^{bc}$ | 14.12$^{a}$ | 13.55$^{abc}$ |
| NCo 334 | 293.33$^{e}$ | 303.91$^{ab}$ | 13.01$^{ab}$ | 13.25$^{bc}$ |
| **CV (%)** | **6.02** | **9.99** | **7.22** | **5.73** |
| **LSD (0.5%)** | **33.38** | **50.69** | **1.56** | **1.26** |

Means with the same letter are not significantly different at P≤0.05.

check variety NCO 334 but they were superior when compared with other varieties in all cuttings (PC, 1st and 2nd ratoon), While B59 250, DB386 60, and COK 30 had got the least value.

Regarding cane thickness, significantly thicker cane was obtained from variety M202 46, of all the cuttings (PC, 1st and 2nd ratoon) followed by B60 163, BO60 349, and B58 230. Taller plants were produced by variety CO 1148 in all the three cuttings followed by variety COK 30. The plant height and cane girth are the major contributing factors for high cane yield (Rehman et al., 1992). Similarly, in this work, the variety M202 46 and CO 1148, which recorded the highest in cane girth and plant height, respectively were grouped in the first category for cane yield ha$^{-1}$ month$^{-1}$ (Table 1).

## Sucrose content, cane yield, and sugar yield

Combined analysis of variance of the data on cane yield, sucrose content and sugar yield revealed that there is an interaction effect of variety and soil on sucrose content and sugar yield for 1st ratoon crop and sucrose content for 2nd ratoon crop, while no interaction in plant cane, cane yield in 1st ratoon and cane yield and sugar yield in 2nd ratoon (Appendix Table 1).

In plant cane, when both soil types were combined, variety N53 216 was rich in sucrose (13.79%) than other varieties followed by DB386 60 (12.63%), in addition except few varieties like B59 250 (7.13%), M202 46 (10.09%) and CO1148 (10.53%) the rest were better in sucrose content than the check variety NCO 334 (11.60%).

In 1st and 2nd ratoon, B59 250 was the least in sucrose content for both soil types, while M202 46 did so in Vertisol only (Tables 2 and 4). Variety N53 216 is also significantly superior in sucrose content than the check variety in Vertisol of 1st and 2nd ratoon (Tables 3 and 5).

Except lower values of DB386 60, B59 250 and B60 163 for plant cane, B59 250 for 1st ratoon and M202 46 and 386 60 for 2nd ratoon, the total tonnage of the cane per hectare per month of sugarcane varieties were not significantly different from the check variety NCO 334 (Table 1, 2 and 4). Variety N53 216 and B58 230 got the highest ton per hectare per month cane yield consistently over the three cuttings (Table 1, 2 and 4). Same wise when data for both soil types were combined, variety N53 216 outperformed the existing commercial sugarcane varieties in sugar yield (1.48, 1.91, and 1.50 t ha$^{-1}$ month$^{-1}$ for plant cane, 1st ratoon and 2nd ratoon respectively) (TableS 1, 2 and 4). Except DB386 60, B59 250 and B60 163 for plant cane, N53 216 (significantly higher than the check), B59 250 and M202 46 for 1st ratoon and M202 46, DB386 60, B59 250 and B60 163 for 2nd ratoon, the total tonnage of sugar yield per hectare per month of sugarcane varieties were not significantly different from the check variety NCO 334. Variety B59 250 produced the least sugar yield compared to any other variety in all the three cuttings (Table 1, 2 and 4).

Generally, the mean values of cane and sugar yield of the three cuttings indicated that, among the evaluated eleven sugarcane varieties N53 216 and B58 230 were the best performing varieties in both soil types viz. luvisol and vertisol. Beside, BO-60349 and B60/163 were also outstanding sugarcane varieties in sugar yield on luvisol and vertisol, respectively (Table 6). On Luvisol, variety N53 216, BO 60349 and B58 230 gave a sugar yield advantage of 25.7, 5.4 and 4.9% over the check variety

**Table 4.** Pooled mean Performance for 8 traits of 12 sugarcane varieties grown at 2 soil types - 2nd Ratoon.

| Varieties | Tiller no. ('000' ha$^{-1}$) | No. of inter-node | Cane height (cm) | Cane thickness (mm) | Millable cane ('000' ha$^{-1}$) | Cane yield (t ha$^{-1}$ month$^{-1}$) | Sucrose % Cane | Sugar yield (t ha$^{-1}$ month$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| B58 230 | 302.82$^{bcde}$ | 22.9$^{cd}$ | 230.7$^{d}$ | 24.6$^{b}$ | 130.35$^{de}$ | 10.22$^{ab}$ | 12.43$^{de}$ | 1.27$^{bc}$ |
| N53 216 | 345.57$^{a}$ | 23.9$^{bc}$ | 233.1$^{d}$ | 24.2$^{b}$ | 154.82$^{b}$ | 10.78$^{a}$ | 13.86$^{a}$ | 1.50$^{a}$ |
| N52 219 | 245.57$^{f}$ | 25.9$^{a}$ | 265.3$^{b}$ | 22.8$^{c}$ | 127.18$^{de}$ | 9.38$^{abc}$ | 12.95$^{bcd}$ | 1.22$^{bcd}$ |
| M202 46 | 307.53$^{bcd}$ | 24.6$^{abc}$ | 256.2$^{bc}$ | 29.2$^{a}$ | 80.92$^{g}$ | 8.32$^{c}$ | 12.07$^{e}$ | 1.02$^{d}$ |
| CP47 193 | 289.94$^{cde}$ | 25.4$^{ab}$ | 259.6$^{bc}$ | 19.2$^{e}$ | 163.74$^{b}$ | 8.77$^{bc}$ | 12.63$^{cde}$ | 1.11$^{bcd}$ |
| DB386 60 | 292.36$^{cde}$ | 22.1$^{d}$ | 236.6$^{d}$ | 22.3$^{c}$ | 121.15$^{ef}$ | 8.00$^{c}$ | 13.09$^{bc}$ | 1.05$^{d}$ |
| B59 250 | 340.34$^{ab}$ | 21.9$^{d}$ | 285.5$^{a}$ | 18.4$^{e}$ | 187.19$^{a}$ | 8.60$^{bc}$ | 8.26$^{f}$ | 0.71$^{e}$ |
| COK 30 | 326.84$^{abc}$ | 21.9$^{d}$ | 288.6$^{a}$ | 21.1$^{d}$ | 138.18$^{cd}$ | 9.59$^{abc}$ | 12.65$^{cde}$ | 1.21$^{bcd}$ |
| B60 163 | 265.80$^{ef}$ | 22.9$^{cd}$ | 256.1$^{bc}$ | 25.2$^{b}$ | 108.39$^{f}$ | 8.62$^{bc}$ | 12.28$^{e}$ | 1.06$^{cd}$ |
| CO 1148 | 315.46$^{abcd}$ | 25.0$^{ab}$ | 292.2$^{a}$ | 22.8$^{c}$ | 119.65$^{ef}$ | 10.25$^{ab}$ | 12.06$^{e}$ | 1.23$^{bcd}$ |
| BO 60349 | 284.77$^{de}$ | 24.0$^{bc}$ | 241.9$^{cd}$ | 25.1$^{b}$ | 114.43$^{ef}$ | 8.72$^{bc}$ | 13.29$^{b}$ | 1.15$^{bcd}$ |
| NCo 334 | 306.44$^{bcd}$ | 24.4$^{abc}$ | 233.3$^{d}$ | 22.5$^{c}$ | 151.09$^{bc}$ | 10.10$^{ab}$ | 12.95$^{bcd}$ | 1.31$^{ab}$ |
| **LSD (0.5%)** | **33.67** | **1.55** | **17.25** | **1.10** | **15.21** | **1.43** | **0.525** | **0.19** |
| **Soils (S)** | | | | | | | | |
| Luvisol | 325.49$^{a}$ | 20.97$^{b}$ | 263.3$^{a}$ | 24.02$^{a}$ | 122.17$^{b}$ | 9.89$^{a}$ | 12.22$^{b}$ | 1.22$^{a}$ |
| Vertisol | 278.42$^{b}$ | 26.48$^{a}$ | 249.9$^{b}$ | 22.21$^{b}$ | 144.01$^{a}$ | 8.67$^{b}$ | 12.53$^{a}$ | 1.09$^{b}$ |
| **LSD (0.5%)** | 13.75 | 0.63 | 7.04 | 0.45 | 6.21 | 0.58 | 0.214 | 0.08 |
| **V x S** | **NS** | **NS** | ****** | **NS** | ***** | **NS** | ******* | **NS** |
| **CV (%)** | 9.59 | 5.62 | 5.78 | 4.08 | 9.83 | 13.24 | 3.65 | 14.31 |

Means with the same letter are not significantly different at P≤0.05.

NCo 334, respectively. On the other hand, variety N53 216, B60 163 and B58 230 gave a sugar yield advantage of 27.9, 2.1 and 1.5% over the check variety NCo 334 on Vertisol. According to Worku and Chinawong (2006) different performances of the same variety on distinct two soil types might have been attributed to the differential response potential to the environment in which it was grown. In agreement with this result, Dillewijn (1952) and Kakde (1985) reported that the differences in the ability of a variety to extract nutrients from different soil types affected its potential to grow under a given soil condition. Better performance of some varieties on both soil types could perhaps indicate their wide adaptation to different soil types.

## CONCLUSION AND RECOMMENDATIONS

Result clearly showed that variety N53 216 and B58 230 produced higher mean cane and sugar yields (ton ha$^{-1}$ month$^{-1}$) than the check variety NCo 334 in both Vertisol and Luvisol fields of Finchaa.

Besides, variety B60 163 responded well in vertisol in either of cane and sugar yield unlike BO60349 which was better in Luvisol. Whereas variety B59 250 in Luvisol and variety N52 219, B59 250, and COK 30 in Vertisol were significantly inferior in cane and sugar yield. Further, variety M202/46 also significantly inferior in sugar yield than the check variety NCo 334 in Vertisol. On Luvisol, variety N53 216, BO 60349 and B58 230 gave a sugar yield advantage of 25.7, 5.4 and 4.9% over the check variety NCo 334, respectively. On the other hand, variety N53 216, B60 163 and B58 230 gave a sugar yield advantage of 27.9, 2.1 and 1.5% over the check variety NCo 334 on Vertisol. In all the three cuttings (plant cane, first ratoon and second ratoon) apart from other varieties N53 216 had

**Table 5.** The effect of variety and soil type on cane height, millable cane and sucrose content - 2nd ratoon.

| Varieties | Cane height (cm) | | Millable cane ('000' ha$^{-1}$) | | Sucrose % cane | |
|---|---|---|---|---|---|---|
| | Luvisol | Vertisol | Luvisol | Vertisol | Luvisol | Vertisol |
| B58 230 | 229.4$^{e}$ | 232.1$^{c}$ | 120.92$^{bcd}$ | 139.78$^{de}$ | 12.19$^{c}$ | 12.66$^{bc}$ |
| N53 216 | 237.4$^{de}$ | 228.8$^{c}$ | 140.77$^{b}$ | 168.86$^{bc}$ | 13.85$^{a}$ | 13.86$^{a}$ |
| N52 219 | 282.0$^{b}$ | 248.6$^{bc}$ | 127.59$^{bc}$ | 126.78$^{ef}$ | 13.15$^{ab}$ | 12.74$^{bc}$ |
| M202 46 | 271.4$^{bc}$ | 241.0$^{bc}$ | 71.84$^{f}$ | 90.00$^{g}$ | 12.42$^{bc}$ | 11.72$^{d}$ |
| CP47 193 | 273.3$^{bc}$ | 245.8$^{bc}$ | 140.12$^{b}$ | 187.36$^{ab}$ | 13.05$^{abc}$ | 12.21$^{cd}$ |
| DB386 60 | 235.7$^{de}$ | 237.5$^{c}$ | 104.37$^{de}$ | 137.93$^{de}$ | 12.34$^{bc}$ | 13.83$^{a}$ |
| B59 250 | 273.1$^{bc}$ | 297.9$^{a}$ | 174.49$^{a}$ | 199.89$^{a}$ | 9.16$^{e}$ | 7.36$^{e}$ |
| COK 30 | 313.8$^{b}$ | 263.3$^{b}$ | 128.48$^{bc}$ | 147.87$^{cde}$ | 12.41$^{bc}$ | 12.89$^{bc}$ |
| B60 163 | 262.0$^{bcd}$ | 250.3$^{bc}$ | 86.21$^{ef}$ | 130.57$^{ef}$ | 11.21$^{d}$ | 13.35$^{ab}$ |
| CO 1148 | 292.6$^{ab}$ | 291.8$^{a}$ | 112.42$^{cd}$ | 126.90$^{ef}$ | 11.34$^{d}$ | 12.78$^{bc}$ |
| BO 60349 | 246.3$^{cde}$ | 237.6$^{c}$ | 116.67$^{cd}$ | 112.18$^{f}$ | 12.54$^{bc}$ | 14.05$^{a}$ |
| NCo 334 | 242.2$^{cde}$ | 224.5$^{c}$ | 142.19$^{b}$ | 160.00$^{cd}$ | 13.02$^{abc}$ | 12.89$^{bc}$ |
| **CV (%)** | **6.35** | **5.35** | **10.07** | **9.03** | **3.93** | **3.35** |
| **LSD(0.5%)** | **28.32** | **22.62** | **20.84** | **22.01** | **0.81** | **0.71** |

Means with the same letter are not significantly different at P≤0.05.

**Table 6.** The 3 cutting mean values of cane and sugar yield of 12 sugarcane varieties grown at 2 soil types.

| Var. | Cane Yield (t ha$^{-1}$ month$^{-1}$) | | | | | | | | Sugar Yield (t ha$^{-1}$ month$^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Luvisol | | | | Vertisol | | | | Luvisol | | | | Vertisol | | | |
| | C - I | C - II | C - III | Mean | C - I | C - II | C - III | Mean | C - I | C - II | C - III | Mean | C - I | C - II | C - III | Mean |
| B58 230 | 11.48 | 11.21 | 11.26 | 11.32$^{AB}$ | 12.55 | 13.29 | 9.18 | 11.67$^{AB}$ | 1.344 | 1.524 | 1.384 | 1.417$^{B}$ | 1.470 | 1.702 | 1.162 | 1.445$^{B}$ |
| N53 216 | 13.43 | 13.47 | 10.66 | 12.52$^{A}$ | 13.76 | 13.70 | 10.91 | 12.79$^{A}$ | 1.822 | 1.789 | 1.483 | 1.698$^{A}$ | 1.927 | 2.021 | 1.518 | 1.822$^{A}$ |
| N52 219 | 9.83 | 11.22 | 10.09 | 10.38$^{BC}$ | 8.59 | 10.20 | 8.68 | 9.16$^{DE}$ | 1.208 | 1.368 | 1.332 | 1.303$^{BC}$ | 1.089 | 1.342 | 1.111 | 1.181$^{CD}$ |
| M202/46 | 10.04 | 10.01 | 9.46 | 9.83$^{CD}$ | 11.11 | 12.15 | 7.19 | 10.15$^{BCD}$ | 1.036 | 1.256 | 1.195 | 1.162$^{C}$ | 1.085 | 1.397 | 0.848 | 1.110$^{D}$ |
| CP47/193 | 10.33 | 11.29 | 9.60 | 10.41$^{BC}$ | 11.24 | 12.38 | 7.94 | 10.52$^{BCD}$ | 1.246 | 1.231 | 1.261 | 1.246$^{BC}$ | 1.378 | 1.771 | 0.968 | 1.372$^{BC}$ |
| DB386/ 60 | 10.77 | 9.99 | 7.94 | 9.57$^{CD}$ | 10.28 | 11.26 | 8.06 | 9.87$^{CD}$ | 1.431 | 1.336 | 0.983 | 1.250$^{BC}$ | 1.225 | 1.548 | 1.114 | 1.295$^{BCD}$ |
| B59 250 | 6.52 | 9.81 | 8.81 | 8.38$^{D}$ | 7.67 | 8.29 | 8.39 | 8.12$^{E}$ | 0.478 | 0.998 | 0.794 | 0.756$^{D}$ | 0.563 | 0.712 | 0.623 | 0.633$^{E}$ |
| COK 30 | 10.38 | 10.47 | 11.06 | 10.64$^{BC}$ | 9.75 | 10.29 | 8.12 | 9.39$^{DE}$ | 1.186 | 1.384 | 1.384 | 1.318$^{BC}$ | 1.145 | 1.377 | 1.042 | 1.188$^{CD}$ |
| B60 163 | 12.66 | 10.36 | 8.32 | 10.45$^{BC}$ | 12.87 | 13.21 | 8.91 | 11.66$^{AB}$ | 1.505 | 1.432 | 0.936 | 1.291$^{BC}$ | 1.417 | 1.765 | 1.181 | 1.454$^{B}$ |
| CO 1148 | 12.06 | 10.80 | 10.88 | 11.25$^{AB}$ | 11.02 | 12.54 | 9.62 | 11.06$^{BC}$ | 1.259 | 1.376 | 1.243 | 1.293$^{BC}$ | 1.147 | 1.623 | 1.215 | 1.328$^{BC}$ |
| BO 60349 | 12.97 | 10.16 | 9.82 | 10.98$^{ABC}$ | 12.80 | 10.61 | 7.62 | 10.34$^{BCD}$ | 1.597 | 1.432 | 1.244 | 1.424$^{B}$ | 1.422 | 1.440 | 1.063 | 1.308$^{BCD}$ |
| NCo 334 | 10.46 | 11.02 | 10.74 | 10.74$^{BC}$ | 11.36 | 13.00 | 9.45 | 11.27$^{ABC}$ | 1.204 | 1.436 | 1.413 | 1.351$^{BC}$ | 1.328 | 1.731 | 1.212 | 1.424$^{B}$ |

C-I, Cutting one; C-II, cutting two; C-III, cutting three.

got significantly higher amount of cane yield, sucrose percent cane and sugar yield in both soil types, that is, Vertisol and Luvisol. Therefore the present finding clearly indicates that variety N53 216 was performing better than the rest test varieties including the check in both soil types. Furthermore, except for sugarcane varieties B59 250 in both soil types and varieties N52 219, M202/46 and COK 30 in luvisol the rest were not significantly different from the check variety NCo 334. Hence, the sugarcane varieties N53 216, B58 230, BO 60349, B60 163, and CP47/193, DB386/60, and CO 1148 in both soil; whereas N52 219, M202/46 and COK 30 only in Luvisol were selected to be verified further on large commercial fields at Finchaa Sugar Estate.

## Conflicts of Interests

The authors have not declared any conflict of interests.

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## APPENDIX

**Table 1.** Combined analysis and their significance from analysis of variance for different parameters of 12 sugarcane varieties grown at 2 soil types (Luvisol and Vertisol).

| Source of variation | DF | Sprouting (%) | Tiller no. per ha | Number of inter-node | Cane height | Cane thickness | Millable cane | Cane yield | Sucrose % cane | Sugar yield |
|---|---|---|---|---|---|---|---|---|---|---|
| **Plant cane** | | | | | | | | | | |
| Soil (S) | 1 | ** | NS | ** | * | * | *** | *** | NS | ** |
| Varieties (V) | 11 | *** | *** | *** | *** | *** | *** | ** | *** | *** |
| S * V | 11 | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| **First ratoon** | | | | | | | | | | |
| Soil (S) | 1 | - | *** | *** | *** | *** | *** | ** | NS | *** |
| Varieties (V) | 11 | - | *** | ** | *** | *** | *** | *** | *** | *** |
| S * V | 11 | - | * | NS | NS | NS | NS | NS | *** | * |
| **Second ratoon** | | | | | | | | | | |
| Soil | 1 | - | *** | *** | *** | *** | *** | *** | ** | ** |
| Varieties | 11 | - | *** | *** | *** | *** | *** | ** | *** | *** |
| S * V | 11 | - | NS | NS | ** | NS | * | NS | *** | NS |

NB: NS means not significant, * means significant at 0.01% significant level, ** means significant at 1% significant level, ***means significant at 5% significant level

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*Full Length Research Paper*

# Predicting grain yields of maize in an Ultisol amended with organic wastes using modified productivity index in Abakaliki, Southeastern Nigeria

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**Predicting grain yields of maize in soil amended with organic wastes using modified productivity index was studied for three cropping seasons. Soil samples for determination of productivity index and individual productivity indicators for prediction of grain yields of maize were collected from 0 15, 15-30, 30-45, and 45-60 cm depths. Ascribed sufficiency value was assigned to each productivity indicator of bulk density, available water capacity (AWC) pH and depth of rooting zone (DRZ) which were used to calculate productivity index (PI) for each amendment. Highest predictions of grain yields of maize were obtained for sawdust PI=0.39 and grain yield of maize-2.30 t ha$^{-1}$ in 2013 and burnt rice mill waste PI=0.39 and grain yield of maize=2.30 t ha$^{-1}$ and PI=0.37 and grain yield of maize=2.25 t ha$^{-1}$ in 2014 and 2015 cropping seasons, respectively. The prediction of organic wastes for grain yields of maize is as follows BRMW>SD>URMW>C. Calculated productivity index (CPI) predicted highly significant (r=0.92 and r$^2$=0.84) grain yields of maize. Bulk density more than AWC and pH predicted highly significant (r=0.95 and r$^2$ = 0.89) grain yield of maize.**

**Key words:** Amended, grain yields of maize, organic wastes, predicting, productivity index.

## INTRODUCTION

Maize (*Zea mays* L.) is an important food and industrial cereal that has contributed greatly to the growth of many developing countries (FAO, 1998; Ande et al., 2008). It belongs to the grain under the family Graminae and class of cereals that thrive under a wide range of environmental conditions (Mbah et al., 2009), although, grain yields are affected by nature and physical conditions as well as nutrients storage of the soil.

Physicochemical condition of a soil is fundamental to its productivity. For instance, soil properties have high degree of relationship with its productivity and crop yield (Wallace and Wallace, 2011; Nnaji, 2009). Corroborating (Follet and Stewart, 1985) noted that relationship existed between soil properties and soil's capacity for producing plants or soil productivity. Anikwe (2000) had related bulk density, available water capacity, depth of rooting zone, pH and generally nutrient storage to soil productivity.

Thus, soil productivity which is expressed in form of

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crop yield can be determined using various approaches. Some of these approaches have been developed in an attempt to numerically relate soil properties to its productivity (Anikwe, 2000). These include Universal Soil Loss Equation (USLE) and Erosion Productivity Impact Calculator (EPIC) (National Soil Erosion and Productivity Research Planning Committee NSEPRPC, 1981). However, a simple numerical index model generally preferred and which has received wide acceptability due to its simplicity and applicability in many soils (Nwite and Alu, 2015; Anikwe 2000) is the productivity index (PI). This model is used widely today for prediction of crop yield. The model is based on the use of physical and chemical properties of soil to predict crop yield and soil productivity.

Modification of productivity index arise because of the need to exclude soil parameters peculiar to a region it was originally conceive and to include those relevant in a new ecological regions where the model is to be currently tested for grain yield predictions. This would not only add value but could increase the models acceptability and applicability in new regions. Productivity index is an algorithm that relates crop yield to rooting depth (Lindstrom et al., 1992), which is controlled by soil environment, while prediction of yield is an estimated projection of crop performance under a specified or set of management system usually expressed in terms of harvestable edible parts. Prediction of future crop yield is essential to make agricultural policy decisions and plan on use of soil in order to sustain both local and national food needs of a nation. Even though, studies have been carried out on soil productivity predictions, little or no research has been documented on prediction of grain yields of maize in the study area. Therefore, it is expected that output from this study would arouse the national psyche towards the need for concerted and sound research on characterization of soils for crop yield predictions. Consequently, critical stakeholders, farmers, policy makers, agronomists and probably other land users might find the result of this work useful. The objectives of this study were to use soil selected physicochemical properties to compute productivity indices for predicting grain yields of maize on soil amended with organic wastes using modified productivity index for three cropping seasons.

## MATERIALS AND METHODS

### Experimental site

The study was carried out at the Teaching and Research Farm of Faculty of Agriculture and Natural Resources Management, Ebonyi State University, Abakaliki. The area is located by Latitude 06°4′N and Longitude 08°65′E in the derived savannah zone of the southeast agro-ecological area of Nigeria (Figure 1). The rainfall pattern is bimodal (April-July and September-November), with a short dry spell in August normally referred to as “August break”. The total annual rainfall in the area ranges from 1500 to 2000 mm, with


**Figure 1.** Map of Abakaliki, Southeast, Nigeria.

a mean of 1,800 mm. At the onset of rainfall, it is torrential and violent, sometimes lasting for one to two hours (Okonkwo and Ogu, 2002). The area is characterized by high temperatures with minimum mean daily temperature of 27°C and maximum mean daily temperature of 31°C throughout the year. Humidity is high (80%) with the lowest (60%) levels occurring during the dry season between December to April, before the rainy season begins (ODNRI, 1989). The underlying geological material in the area is the sedimentary rocks derived from successive marine deposits of the cretaceous and tertiary periods. According to the Federal Department of Agricultural Land Resources (FDALR, 1987) Abakaliki Agricultural zone lies within ‘Asu river group’ and consists of olive brown sandy shales, fine-grained sandstones and mudstones. The soils are shallow with unconsolidated parent materials (shale residuum) within 1 m of the soil surface. The soils of the area are acidic due to mainly heavy and frequent rain falls experienced during rainy seasons and belong to the order ultisol and are classified as Typic Haplustut (FDALR, 1987).

The vegetation of the area is primarily derived savannah, with bush regrowth, and scanty economic trees. The site had history of previous cultivation of yam (*Dioscorea* species) and cassava (*Manihot* species). There is grown of native vegetation such as *Tridax* species*, Odoratum* species*, Aspilla africana, Imperata cylindrica, Panicum maximum, Pennisetum purperum, Sporobulus pyramidalis* and other herbs and shrubs. The common farming practices obtained in the area are continuous cultivation and mixed cropping as a result of population pressure as well as to optimize soil resources. These practices act as “drain” on soil nutrients and cause for low farm outputs often recorded in Abakaliki areas.

### Field methods

#### *Field design/layout and treatment application*

The vegetation was cleared manually using matchet and hoe. The debris left after clearing was removed before seedbed preparation. An area of land that is approximately (0.021 ha) was used for the study. The land was demarcated into plots and replicates. The plots were laid out in Randomized Complete Block Design (RCBD). The plots measured 2 × 2 m with a plot alley of 0.5 spacing. The four replicates were separated by 1 m spaces. The treatments consisted of: control (C), that is, no application of organic wastes; burnt rice mill waste (BRMW) 20 t $ha^{-1}$ equivalent to 8 kg/plot; unburnt rice mill

waste (URMW) 20 t $ha^{-1}$ equivalent to 8 kg/plot; sawdust (SD) 20 t $ha^{-1}$ equivalent to 8 kg/plot.

The treatments namely were burnt rice mill waste (BRMW), fresh or unburnt rice mill waste (URMW) and sawdust (SD) were sourced from the agro-rice mill industry and timber shade market, Abakaliki, respectively. Agro-rice wastes and sawdust are generated from numerous rice mills and timber shades that criss cross the state. These wastes are heaped to form artificial mountains causing environmental nuisance as they are not put to any useful use in the area. Furthermore, they are cheap and available to resource poor farmers. The organic wastes of burnt rice mill waste, unburnt rice mill waste and sawdust were spread on the plots. They were incorporated into the soil during seedbed preparation using traditional hoe. The beds were allowed to age for two weeks after incorporation of treatments before planting the test crop. The treatments were replicated four times to give a total of twenty plots in the study.

Maize seed (suwan-1-SR-hybrid variety) sourced from Ebonyi State Agricultural Development Programme (EBADEP) was planted 2 seeds per hole at 5 cm depth and spacing distance of 25 × 75 cm. Two weeks after emergence (WAE), the plants were thinned down to one plant per hole while lost stands were replaced. Weak plants were rogued out and replaced leaving a plant population of approximately 53, 000 stands per hectare. There was application of NPK (20:10:10) fertilizer at 400 kg $ha^{-1}$ to all the plots two weeks after plant emergence (WAPE). The fertilizer was banded and placed 5 cm away from the maize plants. Weeds were removed at three-weekly intervals up till harvest. In the second year, the procedure was repeated while residual effect was tested in the third year of study without fresh application of treatments.

**Agronomic data**

The cobs were harvested at plant maturity. This was when the husks were dried. The cobs were dehusked and further dried before shelling and grain yield determined at 14% moisture content. Agronomic yield data were taken on twelve tagged plants representation, 25% of plant population per plot.

***Soil sampling***

Initial soil samples were collected from the 0 to 20 cm depth using auger at different points in the study site before application of organic wastes and cultivation. The auger samples were composited and used for routine laboratory analysis. Core and auger samples were collected at 0 to 15, 15 to 30, 30 to 45 and 45 to 60 cm depths in each plot and used for soil productivity evaluation. Core samples were used to determine some soil physical properties while auger samples were air-dried at room temperature (about 26°C) and passed through a 2 mm sieve. These were used for pH determination.

***Laboratory determination***

Dry bulk density was determined as described by Blake and Hartge (1986). Particle size distribution was determined by the hydrometer method as described by Gee and Or (2002). The result from particle size distribution was reported as percentage sand, silt and clay respectively.

Moisture retained at -10 and -1500 $Kp_a$ matric potentials were estimated based on the saturation water percentage ($S_p$) models of Mbagwu and Mbah (1998). The models are: available water capacity (AWC) was computed as the difference between moisture retained at 10 and 100 $kp_a$ matric potentials, where

$$\Theta.01\ (FC) = -6.22 + 0.79\ (S_p) \quad (1)$$

$$\Theta\ .100 = -\ 10.95 + 0.65\ (S_p) \quad (2)$$

$$\Theta\ .15\ (PWP) = -\ 8.65 + 0.51\ (S_p) \quad (3)$$

where FC is the field capacity, $S_p$ is the saturation percentage, and PWP is permanent wilting point.

The pH determination of the soil was in duplicates both in distilled water and in 0.1N KCL solution using a soil/water ratio 1:2.5. After stirring for 30 min, the pH values were read off using a Beckman zeromatic pH meter (Peech, 1995). The total nitrogen was determined using the micro-Kjedhal distillation method of Bremner (1996). The ammonia from the digestion was distilled with 45% NaOH into 2.5% boric acid and determined by titrating with 0.05N KCL. Available phosphorus determination was done according to by the Bray-2 method as described in by Page et al. *(*1982). This method involved weighing 2 g of soil sample into a test tube. 20 ml of 0.03 $NH_4F$ in 0.1N HCL was added to the sample of soil in the test tube. Then, the test tube was closed and shook for a minute. It was allowed to settle and filtered. 1 ml of the filtrate was pipetted into a 50 ml of volumetric flask. 7 ml of distilled water and 1 ml of $NH_4$ molybdate and 1 ml of ascorbic acid were added to the sample. The flask was made up to the mark with distilled water and allowed to stand for 15 min before taking the reading using 608 filter paper.

The available phosphorus was read off from the standard curve obtained from optical density using a colorimeter. Organic carbon determination was done by using the method described by Nelson and Sommer (1982). Calcium (Ca) and magnesium (Mg) were determined by titration method (Mba, 2004). Cation exchange capacity (CEC) was determined by ammonium acetate ($NH_4OC$) displacement method (Jackson, 1958).

The burnt rice mill waste, unburnt or fresh rice mill waste and sawdust organic wastes were analyzed for calcium (Ca), magnesium (Mg), nitrogen (N), phosphorus (P), organic carbon (OC) and C:N ratio using the method of Juo (1983).

**Soil productivity index and its modification**

The Pierce et al. (1983) productivity index is expressed thus:

$$PI = \sum_{i=1}^{r} (A_i x B_i x C_i x D_i x E_i x Wf_i) \quad (4)$$

where PI is the productivity index, Ai is the sufficiency for available water capacity for the ith soil layer, $B_i$ is the sufficiency for aeration for the ith soil layer, $C_i$is the sufficiency for pH for the ith soil layer, $D_i$ is the sufficiency for bulk density for the ith soil layer, $E_i$ is the sufficiency for electrical conductivity for the ith soil layer, $Wf_i$ is the root weighting factor, and r is the number of horizons in the rooting zone

***Modified Pierce et al. (1983) productivity index***

Pierce et al. (1983) productivity index model as used in this work was modified to exclude sufficiency for aeration since it could be predicted from bulk density and sufficiency for electrical conductivity which is not common under humid conditions. Hence, the modified productivity index.

$$PI_M = \sum_{i=1}^{r} (A_i x C_i x D_i x Wf_i) \quad (5)$$

where $PI_M$ is the modified productivity index, Ai is the sufficiency for

**Table 1.** Some properties of soil at initiation of study.

| Soil properties | Unit | Value |
|---|---|---|
| pH KCL | - | 5.1 |
| Organic carbon | % | 1.84 |
| Nitrogen | % | 0.16 |
| Available P | $mgkg^{-1}$ | 4.70 |
| Calcium | $cmolkg^{-1}$ | 5.20 |
| Magnesium | $cmolkg^{-1}$ | 3.80 |
| Cation exchange capacity | $cmolkg^{-1}$ | 10.3 |

**Table 2.** Some properties of amendment materials.

| Treatment | Parameter | Unit | Value |
|---|---|---|---|
| Burnt rice mill waste | Organic carbon | % | 6.92 |
| | Nitrogen | % | 0.30 |
| | Phosphorus | $mgkg^{-1}$ | 14.00 |
| | Calcium | $cmolkg^{-1}$ | 1.17 |
| | Mg | $cmolkg^{-1}$ | 0.27 |
| | C:N | - | 23 |
| Unburnt rice mill waste | Organic carbon | % | 16.39 |
| | Nitrogen | % | 0.48 |
| | Phosphorus | $mgkg^{-1}$ | 7.00 |
| | Calcium | $cmolkg^{-1}$ | 0.50 |
| | Mg | $cmolkg^{-1}$ | 0.12 |
| | C:N | - | 34 |
| Sawdust | Organic carbon | % | 8.99 |
| | Nitrogen | % | 0.28 |
| | Phosphorus | $mgkg^{-1}$ | 3.00 |
| | Calcium | $cmolkg^{-1}$ | 0.30 |
| | Mg | $cmolkg^{-1}$ | 0.10 |
| | C:N | - | 32 |

available water capacity for the ith soil layer, $C_i$ is the sufficiency for pH for the ith soil layer, $D_i$ is the sufficiency for bulk density for the ith soil layer, $Wf_i$ is the root weighting factor, and r is the number of horizons in the rooting zone.

#### Data analysis

The data collected from this experiment were subjected to Statistical Analysis System (SAS, 1985) method. Significant treatment effect was reported at 5% probability level. Correlation and regression analysis according to Steel and Torrie (1980) were used to determine the relationship between soil productivity indicators and yield data.

## RESULTS

Table 1 shows some properties of soil at initiation of study. The pH in KCL was 5.1. The respective percentage organic carbon and nitrogen were 1.84 and 0.16%. Available phosphorus was low with a value of 4.70 $mgkg^{-1}$. Calcium and magnesium of the soil were 5.20 and 3.80 $cmolkg^{-1}$ in the exchange complex of soil. The cation exchange capacity was 10.3 $cmolkg^{-1}$.

Some properties of amendment materials are shown in Table 2. Organic carbon and total N ranged from 6.92 to 16.39 and 0.28 to 0.48%, while available phosphorus ranged from 3.00 to 14.00 $mgkg^{-1}$ and calcium and magnesium ranged from 0.30 to 1.17 $cmolkg^{-1}$ and 0.10 to 0.27 $cmolkg^{-1}$ in the organic wastes. Carbon-nitrogen ratio for the organic wastes were 23, 32 and 34 for burnt rice mill waste, sawdust and unburnt rice mill waste, respectively.

Tables 3 to 5 show soil properties, ascribed sufficiency values and calculated productivity index for each of the treatments for 2013, 2014 and 2015 cropping seasons.

**Table 3.** Soil properties, ascribed sufficiency value and calculated productivity indices for 2013

| **Control** | **……..Measured property soil……..** | | | | **…….2013 Ascribed sufficiency of soil…….** | | | |
|---|---|---|---|---|---|---|---|---|
| **Soil depth (cm)** | **BD ($mgm^{-3}$)** | **AWC ($cm^{-1}$)[1]** | **pH (kcl)** | **RWF (cm)** | **BD ($mgm^{-3}$)** | **AWC ($cm^{-1}$)** | **pH (kcl)** | **RWF (cm)** |
| 0-15 | 1.66 | 0.17 | 3.5 | 60 | 0.13 | 0.65 | 0.25 | 1.00 |
| 15-30 | 1.68 | 0.18 | 3.6 | 60 | 0.11 | 0.70 | 0.21 | 1.00 |
| 30-45 | 1.70 | 0.19 | 3.3 | 60 | 0.09 | 0.78 | 0.16 | 1.00 |
| 45-60 | 1.78 | 0.20 | 3.0 | 60 | 0.02 | 0.79 | 0.14 | 1.00 |
| PI | | | | | 0.28 | | | |
| **URMW** | **……..…..Measured property of soil…….……** | | | | **…..…….Ascribed sufficiency of soil……..** | | | |
| **Soil depth (cm)** | **BD ($mgm^{-3}$)** | **AWC ($cm^{-1}$)** | **pH (kcl)** | **RWF (cm)** | **BD ($mgm^{-3}$)** | **AWC ($cm^{-1}$)** | **pH (kcl)** | **RWF (cm)** |
| 0-15 | 1.51 | 0.18 | 4.0 | 60 | 1.00 | 0.70 | 0.47 | 1.00 |
| 15-30 | 1.62 | 0.19 | 3.7 | 60 | 0.96 | 0.78 | 0.34 | 1.00 |
| 30-45 | 1.64 | 0.20 | 3.5 | 60 | 0.92 | 0.79 | 0.25 | 1.00 |
| 45-60 | 1.66 | 0.21 | 3.5 | 60 | 0.81 | 0.80 | 0.25 | 1.00 |
| PI | | | | | 0.36 | | | |
| **BRMW** | **……..Measured property of soil…….** | | | | **…..……Ascribed sufficiency of soil……..….** | | | |
| **Soil depth (cm)** | **BD ($mgm^{-3}$)** | **AWC ($cm^{-1}$)** | **pH (kcl)** | **RWF (cm)** | **BD ($mgm^{-3}$)** | **AWC ($cm^{-1}$)** | **pH (kcl)** | **RWF (cm)** |
| 0-15 | 1.62 | 0.18 | 3.7 | 60 | 0.60 | 0.70 | 0.34 | 1.00 |
| 15-30 | 1.64 | 0.18 | 3.6 | 60 | 0.58 | 0.70 | 0.30 | 1.00 |
| 30-45 | 1.65 | 0.19 | 3.5 | 60 | 0.50 | 0.78 | 0.25 | 1.00 |
| 45-60 | 1.66 | 0.20 | 3.4 | 60 | 0.48 | 0.79 | 0.16 | 1.00 |
| PI | | | | | 0.38 | | | |
| **SD** | **…….…..Measured property of soil……** | | | | **…….Ascribed sufficiency of soil……..** | | | |
| **Soil depth (cm)** | **BD ($mgm^{-3}$)** | **AWC ($cm^{-1}$)** | **pH (kcl)** | **RWF (cm)** | **BD ($mgm^{-3}$)** | **AWC ($cm^{-1}$)** | **pH (kcl)** | **RWF (cm)** |
| 0-15 | 1.63 | 0.18 | 3.7 | 60 | 0.61 | 0.70 | 0.34 | 1.00 |
| 15-30 | 1.64 | 0.18 | 3.6 | 60 | 0.58 | 0.70 | 0.30 | 1.00 |
| 30-45 | 1.65 | 0.19 | 3.5 | 60 | 0.50 | 0.78 | 0.25 | 1.00 |
| 45-60 | 1.65 | 0.20 | 3.4 | 60 | 0.50 | 0.79 | 0.16 | 1.00 |
| PI | | | | | 0.39 | | | |

C-control, B- burnt rice husk dust, U- Unburnt rice husk dust, S- Sawdust, PI- productivity index.

**Table 4.** Soil properties, ascribed sufficiency value and calculated productivity indices for 2014

| **Control** | **……..Measured property of soil……** | | | | **……2014 Ascribed sufficiency of soil……** | | | |
|---|---|---|---|---|---|---|---|---|
| **Soil depth (cm)** | **BD ($mgm^{-3}$)** | **AWC ($cm^{-1}$)** | **pH (kcl)** | **RWF (cm)** | **BD ($mgm^{-3}$)** | **AWC ($cm^{-1}$)** | **pH (kcl)** | **RWF (cm)** |
| 0-15 | 1.67 | 0.16 | 3.5 | 60 | 0.12 | 0.60 | 0.25 | 1.00 |
| 15-30 | 1.66 | 0.18 | 3.5 | 60 | 0.05 | 0.70 | 0.25 | 1.00 |
| 30-45 | 1.80 | 0.19 | 3.3 | 60 | 0.04 | 0.78 | 0.16 | 1.00 |
| 45-60 | 1.80 | 0.20 | 3.0 | 60 | 0.04 | 0.79 | 0.14 | 1.00 |
| PI | | | | | 0.26 | | | |
| **URMW** | **……Measured property of soil……** | | | | **……Ascribed sufficiency of soil……** | | | |
| **Soil depth (cm)** | **BD ($mgm^{-3}$)** | **AWC ($cm^{-1}$)** | **pH (kcl)** | **RWF (cm)** | **BD ($mgm^{-3}$)** | **AWC ($cm^{-1}$)** | **pH (kcl)** | **RWF (cm)** |
| 0-15 | 1.78 | 0.16 | 4.3 | 60 | 0.10 | 0.65 | 0.64 | 1.00 |
| 15-30 | 1.79 | 0.18 | 4.0 | 60 | 0.11 | 0.70 | 0.47 | 1.00 |
| 30-45 | 1.80 | 0.18 | 4.0 | 60 | 0.04 | 0.70 | 0.47 | 1.00 |
| 45-60 | 1.83 | 0.18 | 3.7 | 60 | 0.01 | 0.70 | 0.34 | 1.00 |
| PI | | | | | 0.36 | | | |

**Table 4.** Contd.

| BRMW | ……Measured property of soil…… | | | | ……Ascribed sufficiency of soil…… | | | |
|---|---|---|---|---|---|---|---|---|
| Soil depth (cm) | BD ($mgm^{-3}$) | AWC ($cm^{-1}$) | pH (kcl) | RWF (cm) | BD ($mgm^{-3}$) | AWC ($cm^{-1}$) | pH (kcl) | RWF (cm) |
| 0-15 | 1.78 | 0.17 | 4.4 | 60 | 0.10 | 0.65 | 0.62 | 1.00 |
| 15-30 | 1.78 | 0.19 | 4.1 | 60 | 0.10 | 0.78 | 0.47 | 1.00 |
| 30-45 | 1.84 | 0.20 | 4.0 | 60 | 0.01 | 0.79 | 0.47 | 1.00 |
| 45-60 | 1.80 | 0.20 | 4.0 | 60 | 0.01 | 0.79 | 0.47 | 1.00 |
| PI | | | | | 0.39 | | | |
| **SD** | **……Measured property of soil……** | | | | **……Ascribed sufficiency of soil……** | | | |
| Soil depth (cm) | BD ($mgm^{-3}$) | AWC ($cm^{-1}$) | pH (kcl) | RWF (cm) | BD ($mgm^{-3}$) | AWC ($cm^{-1}$) | pH (kcl) | RWF (cm) |
| 0-15 | 1.74 | 0.18 | 3.7 | 60 | 0.10 | 0.70 | 0.34 | 1.00 |
| 15-30 | 1.78 | 0.19 | 3.6 | 60 | 0.80 | 0.78 | 0.30 | 1.00 |
| 30-45 | 1.80 | 0.19 | 3.5 | 60 | 0.08 | 0.78 | 0.25 | 1.00 |
| 45-60 | 1.80 | 0.20 | 3.5 | 60 | 0.07 | 0.80 | 0.25 | 1.00 |
| PI | | | | | 0.34 | | | |

C-control, B- burnt rice husk dust, U- Unburnt rice husk dust, S- Sawdust, PI- productivity index.

**Table 5.** Soil properties, ascribed sufficiency values and calculated productivity indices for 2015.

| Control | ……Measured property of soil…… | | | | ……2015 Ascribed sufficiency of soil…… | | | |
|---|---|---|---|---|---|---|---|---|
| Soil depth (cm) | BD ($mgm^{-3}$) | AWC ($cm^{-1}$ | pH (kcl) | RWF (cm) | BD ($mgm^{-3}$) | AWC ($cm^{-1}$ | pH (kcl) | RWF (cm) |
| 0-15 | 1.80 | 0.16 | 4.3 | 60 | 0.08 | 0.60 | 0.61 | 1.00 |
| 15-30 | 1.82 | 0.17 | 4.0 | 60 | 0.05 | 0.65 | 0.47 | 1.00 |
| 30-45 | 1.85 | 0.17 | 3.8 | 60 | 0.04 | 0.65 | 0.38 | 1.00 |
| 45-60 | 1.87 | 0.18 | 3.6 | 60 | 0.03 | 0.70 | 0.30 | 1.00 |
| PI | | | | | 0.21 | | | |
| **URMW** | **……Measured property of soil……** | | | | **……Ascribed sufficiency of soil……** | | | |
| Soil depth (cm) | BD ($mgm^{-3}$) | AWC ($cm^{-1}$) | pH (kcl) | BD ($mgm^{-3}$) | AWC ($cm^{-1}$) | pH (kcl) | BD ($mgm^{-3}$) | AWC ($cm^{-1}$) |
| 0-15 | 1.78 | 0.17 | 4.3 | 60 | 0.10 | 0.64 | 0.64 | 1.00 |
| 15-30 | 1.79 | 0.18 | 4.3 | 60 | 0.11 | 0.70 | 0.64 | 1.00 |
| 30-45 | 1.80 | 0.19 | 4.0 | 60 | 0.08 | 0.78 | 0.47 | 1.00 |
| 45-60 | 1.81 | 0.19 | 3.8 | 60 | 0.07 | 0.78 | 0.38 | 1.00 |
| PI | | | | | 0.35 | | | |
| **BRMW** | **……Measured property of soil……** | | | | **……Ascribed sufficiency of soil……** | | | |
| Soil depth (cm) | BD ($mgm^{-3}$) | AWC ($cm^{-1}$) | pH (kcl) | BD ($mgm^{-3}$) | AWC ($cm^{-1}$) | pH (kcl) | BD ($mgm^{-3}$) | AWC ($cm^{-1}$) |
| 0-15 | 1.80 | 0.17 | 4.4 | 60 | 0.08 | 0.65 | 0.65 | 1.00 |
| 15-30 | 1.82 | 0.18 | 4.3 | 60 | 0.07 | 0.70 | 0.64 | 1.00 |
| 30-45 | 1.83 | 0.18 | 4.3 | 60 | 0.04 | 0.70 | 0.64 | 1.00 |
| 45-60 | 1.85 | 0.19 | 4.0 | 60 | 0.04 | 0.78 | 0.47 | 1.00 |
| PI | | | | | 0.37 | | | |
| **SD** | **……Measured property of soil……** | | | | **……Ascribed sufficiency of soil……** | | | |
| Soil depth (cm) | BD ($mgm^{-3}$) | AWC ($cm^{-1}$) | pH (kcl) | BD ($mgm^{-3}$) | AWC ($cm^{-1}$) | pH (kcl) | BD ($mgm^{-3}$) | AWC ($cm^{-1}$) |
| 0-15 | 1.75 | 0.17 | 4.4 | 60 | 0.12 | 0.65 | 0.65 | 1.00 |
| 15-30 | 1.78 | 0.17 | 4.3 | 60 | 0.10 | 0.65 | 0.64 | 1.00 |
| 30-45 | 1.80 | 0.18 | 4.2 | 60 | 0.08 | 0.70 | 0.65 | 1.00 |
| 45-60 | 1.82 | 0.18 | 4.0 | 60 | 0.06 | 0.70 | 0.47 | 1.00 |
| PI | | | | | 0.32 | | | |

C-control, B- burnt rice husk dust, U- Unburnt rice husk dust, S- Sawdust, PI- productivity index.

**Table 6.** Productivity Index and Grain Yield of Maize.

| Treatment | PI | Grain yield of maize (t ha$^{-1}$) |
|---|---|---|
| Control | 0.28 | 2.20 |
| Control | 0.26 | 2.15 |
| Control | 0.21 | 2.00 |
| BRMW | 0.38 | 2.26 |
| BRMW | 0.39 | 2.30 |
| BRMW | 0.37 | 2.25 |
| SD | 0.39 | 2.30 |
| SD | 0.34 | 2.22 |
| SD | 0.32 | 2.18 |
| URMW | 0.36 | 2.24 |
| URMW | 0.36 | 2.24 |
| URMW | 0.35 | 2.23 |
| Total | 5.14 | 26.57 |
| Mean | 0.42 | 2.21 |

BRMW: Burnt rice mill waste; URMW: unburnt rice mill waste; SD: sawdust.

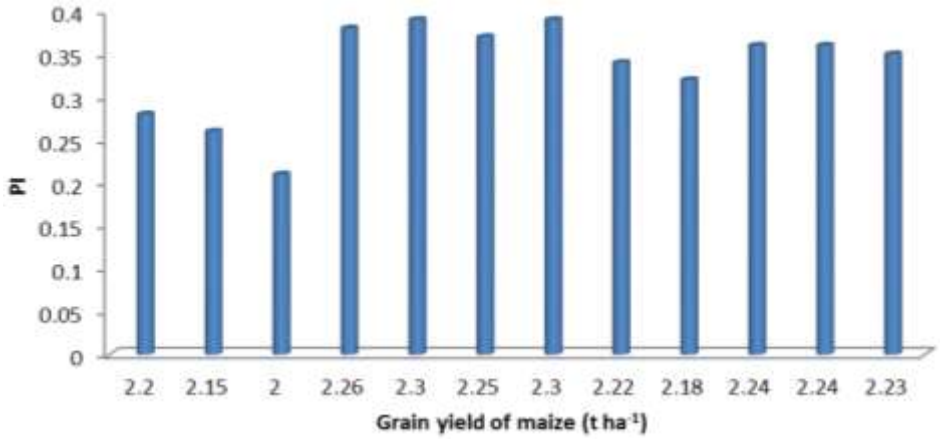


**Figure 2.** Productivity index and grain yield of maize.

The prediction of grain yield of maize was the highest under sawdust amended plot in 2013 cropping season. This was 28 and 30% higher in prediction of grain yields of maize than control and other organic wastes amended plots.

Even though in 2014 cropping season, productivity index (PI) generally declined, BRMW amended plot predicted highest grain yields of maize. These were 33, 8 and 13% higher in prediction of grain yields of maize than control, URMW and SD amended plots. During residual studies, P1 decreased in all the plots. The predictions in grain yields of maize were 7 and 13% lower for control and sawdust amended plots for 2014 cropping season. Similarly, predictions in grain yields of maize were 25, 3, 3 and 18% respectively higher than the values obtained for 2015 cropping season. Ascribed sufficiency value is a dimensionless curve which relates measured soil property to assigned value between 0.0 and 1.0, while calculated productivity index is the value obtained from computation of sufficiency values of individual soil productivity indicators.

The calculated productivity index (CPI) and grain yield of maize is shown in Table 6 and Figure 2. Results showed that mean PI and grain yield of maize were 0.42 and 2.21 t ha$^{-1}$, respectively. The plots supplemented with organic wastes had higher predictions of grain yields of maize than control. Of all the amended plots, the one receiving BRMW amendment had higher prediction of mean of grain yield of maize compared to values obtained for URMW, sawdust and control, respectively. The prediction of grain yields of maize in the treatments followed the trend of BRMW>SD>URMW>C. Generally,

**Table 7.** Relationship between calculated productivity index, individual productivity index and grain yield of maize.

| Dependent crop parameter | Regression model | r | $r^2$ | N=128 |
|---|---|---|---|---|
| PI VS grain yield of maize | Y =1.68x=1.63 | 0.92** | 0.84** | - |
| BD VS grain yield of maize | Y=5.64x-2.16 | 0.95** | 0.89** | - |
| AWC VS grain yield of maize | Y=5.18x+1.24 | 0.75** | 0.57* | - |
| pH VS grain yield of maize | Y=0.34x+0.51 | 0.76** | 0.58* | - |

PI: Productivity index; BD: Bulk density; AWC: Available water capacity. **Significant at 1%, *Significant at 5%, VS: versus, N: number.

**Table 8.** Relationship between individual productivity indicators and calculated productivity index.

| Dependent crop parameter | Regression model | r | $r^2$ | N=128 |
|---|---|---|---|---|
| BD VS Productivity index | Y=2.01x-1.05 | 0.83** | 0.70** | - |
| AWC VS Productivity index | Y=2.77x-0.18 | 0.74** | 0.55ns | - |
| pH VS Productivity index | Y=0.18x-0.57 | 0.75** | 0.56* | - |

BD: Bulk density, AWC: available water capacity, RWF: root weighting factor, *Significant at 5%, **Highly significant at P>0.01, VS: versus, N: number of samples.

prediction of grain yields of maize followed the trends of productivity index.

Table 7 shows relationship between calculated productivity index (CPI) as well as individual productivity indicators (IPI) and grain yield of maize. There were positive and highly significant relationships between Calculated Productivity Index and Individual Productivity Index and grain yield of maize except for $r^2$ relationship between pH and grain yield of maize. Calculated productivity index predicted highly significant (r=0.92 and $r^2$<0.84 at P<0.01) grain yield of maize. The individual productivity indicators predicted highly significant (r=0.95 and $r^2$=0.89 at P<0.01) for bulk density and (r= 0.75 and $r^2$=0.57 at P<0.05) for AWC as well as (r =0.76 and $r^2$ = 0.58 at P<0.05) and grain yields of maize, respectively. In other words, bulk density explained 89 to 95% in soil variations in predicting grain yields of maize. This implies that bulk density rather than AWC, rooting depth or pH influenced soil productivity and grain yields of maize in the soil.

Table 8 shows relationship between calculated productivity index and individual productivity indicators. Result showed positive relationships between Individual productivity indicator and calculated productivity index. Significantly (P<0.01) higher correlation coefficients were obtained between Individual productivity indicators and calculated productivity index.

These were r=0.83 for bulk density and calculated productivity index, r=0.74 for AWC and calculated productivity index and r=0.75 for pH and calculated productivity index, respectively. The coefficient of determination relationship between bulk density and calculated productivity index was significantly ($r^2$=0.70 at P<0.01) higher than the value obtained for AWC and calculated productivity index ($r^2$ =0.55 at P<0.05) and pH and calculated productivity index ($r^2$ = 0.56 at P<0.05).

## DISCUSSION

The soil was strongly acidic in line with FMARD (2002) bench mark for tropical soils. Nitrogen and available phosphorus were rated low in the soil (Enwezor et al., 1981). Cation exchange capacity was rated low according to Asadu and Nweke (1999) bench mark for soils of sub-Sahran Africa. This preliminary investigation indicates that the soil was acidic and of low fertility trend. This could be attributed to inherent properties of tropical soils. Tropical soils had been reported (Asadu et al., 2008) to suffer degradation and poor mineralization of nutrients due to high temperatures.

The Carbon-Nitrogen ratio fell within moderate values as recommended by Biswas and Murkherjee (2008) to enhance decomposition and release of nutrients. Organic carbon and total N in the organic wastes were rated high (Landon, 1991) while available phosphorus was low using critical values established for tropical soils by FMARD (2002). Calcium and magnesium in the organic wastes were rated low (Asadu and Nweke, 1999) according to ratings established for African soils.

Higher prediction of grain yields of maize in organic wastes amended plots compared to control could be attributed to improvement in soil properties due to amended materials. This corroborates the report of Puget et al. (2000) that organic wastes contained valuable materials that improved soil productivity. The decrease in predictions of grain yields of maize in 2014 cropping season and during residual season could be due to continuous cropping on one hand and low impact of residual effect of organic wastes amendment in third

season. Mbah et al. (2009) noted that continuous cropping was depletive on soil nutrients and caused low soil productivity. The superiority of BRMW amendment relative to other amendments in prediction of grain yields of maize could be linked to higher mineralization of nutrients and generally improved soil properties which enhanced predictions of grain yields maize.

Higher predictions of grain yields of maize in plots amended with organic wastes compared to control could be attributed to positive impacts of the amendment materials on soil. It further implies that these materials could improve soil properties and cause increase in grain yield of maize. This study is supported by Mbah et al. (2009) and Adeleye et al. (2010) report that organic wastes amendment improved soil properties and increased its productivity. The generally superior prediction of grain yield of maize obtained in BRMW amended plot could be due to on one hand higher mineralization of nutrients to soil and on the other greater surface area that increased microbial action in degradation of the waste to release nutrients. The results on trend of productivity index and grain yield of maize had been observed by Anikwe (2000) and Nwite and Obi (2008) who reported that grain yields of maize followed the trend of productivity index.

The highly significant prediction of grain yield of maize obtained from calculated productivity index suggests that parameters used to compute productivity index strongly influenced grain yield of maize. This observation was noted by Anikwe and Obi (1999) in their studies that productivity index influenced and determined grain yield of maize. The significantly high prediction of grain yield of maize by productivity index could be further attributed to effectiveness and efficiency of organic wastes amendment in improving soil productivity and grain yield of maize. Superior prediction of grain yield of maize obtained in bulk density compared to AWC, rooting depth and pH could be as a result of its indirect influence on soil moisture status and nutrient storage and supply which also governs crop yield. Furthermore, improvement of soil bulk density could in turn positively influence nutrients storage and generally soil productivity.

The positive relationship between calculated productivity index and individual productivity indicator implies that individual productivity indicator influenced the productivity indices used in predicting the grain yields of maize. This corroborates the findings of Nwite (2013) that individual productivity indicators influenced productivity index in predictions. Significantly higher correlation coefficient obtained between the individual productivity indicator and calculated productivity index tends to suggest that there was synergy among the productivity indicators in promoting prediction of grain yields of maize. Molua and Lambi (2006) reported that available water was the most critical factor determining yield. The highly significant coefficient of determination obtained between bulk density and calculated productivity index compared to AWC and pH and calculated productivity index implies that high soil bulk density could mask influence of AWC and pH on soil productivity and hence reduce crop yield.

## Conclusion

This study had shown that grain yields of maize could be predicted using modified productivity index. Generally, organic wastes amended plots predicted higher grain yields of maize than control. Burnt rice mill waste had superior prediction of grain yields of maize when compared to other wastes amendment. There were positive and significant predictions of grain yields of maize by calculated productivity index and individual productivity indicators. Bulk density influenced prediction of grain yields of maize more than available water capacity, pH and rooting depth. The research indicates that agro-wastes from rice mills and timber shades could be used to improve soil properties for higher productivity. This would be useful alternative way for engaging materials ordinarily abandoned to constitute environmental pollution with its attendant health hazards. Furthermore, modified productivity index gained acceptance and applicability in a new region as it could be used for future projection of food needs of a country. This would help policy makers in moving the country to make provisions in periods of shortfall to avert food crisis.

## Conflict of Interests

The authors have not declared any conflict of interests.

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# Pore size distribution and hydro-physical properties of cohesive horizons treated with anionic polymer

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**Long molecular chain of polymers with active groups, combined with its complexity and flexibility to different environmental conditions provides an interaction of these groups with the mineral soil clays, thereby qualifying the polymers as soil flocculants effective. This study is aimed at evaluating the pore size distribution and hydro-physical properties of soils with cohesive horizons of the coastal plains of Pernambuco State, Brazil, with the application of anionic polyacrylamide (PAM). Thus, three horizons, one cohesive (Bt1) and two non cohesive (E and Bw/Bt) Ultisol were evaluated and, to compare the cohesive horizons, a BA horizon of Oxisol was selected. PAM aqueous solutions (12.5, 50.0 and 100.0 mg $kg^{-1}$) and distilled water (control = 0 mg $kg^{-1}$) were applied in undeformed samples by capillary. Pore volume was evaluated by diameter (macro, meso and micropores), total porosity, saturated hydraulic conductivity (Ksat), soil penetration resistance (PR) and soil-water characteristic retention curve. Polymer solutions reduced Ksat, macroporosity and total porosity of cohesive horizons (micropores dominated). More concentrated solutions increased PR of the Bt1 horizon. The excess negative charges in the system are the main factor for the negative effects of PAM on clay horizons. We hope that less electronegative PAMs may improve the hydro-physical characteristics of cohesive horizons.**

**Key words:** Polyacrylamide, coastal tablelands, soil penetration resistance, negative charges, water retention, macroporosity.

## INTRODUCTION

The low physical quality of soils located in the Coastal Tablelands ecosystem in Brazil is due to subsurface cohesive horizons (Correa et al., 2008). Despite the limited agricultural potential of these soils, this region is among the best agricultural regions in the rankings of agricultural production in Brazil (Souza et al., 2006; Gomes et al., 2012). There are several ecosystems and various functions for which soil can be used, but there is no specific methodology to characterize the soil quality by a universal set of indicators (Bouma, 2002). Thus, soil

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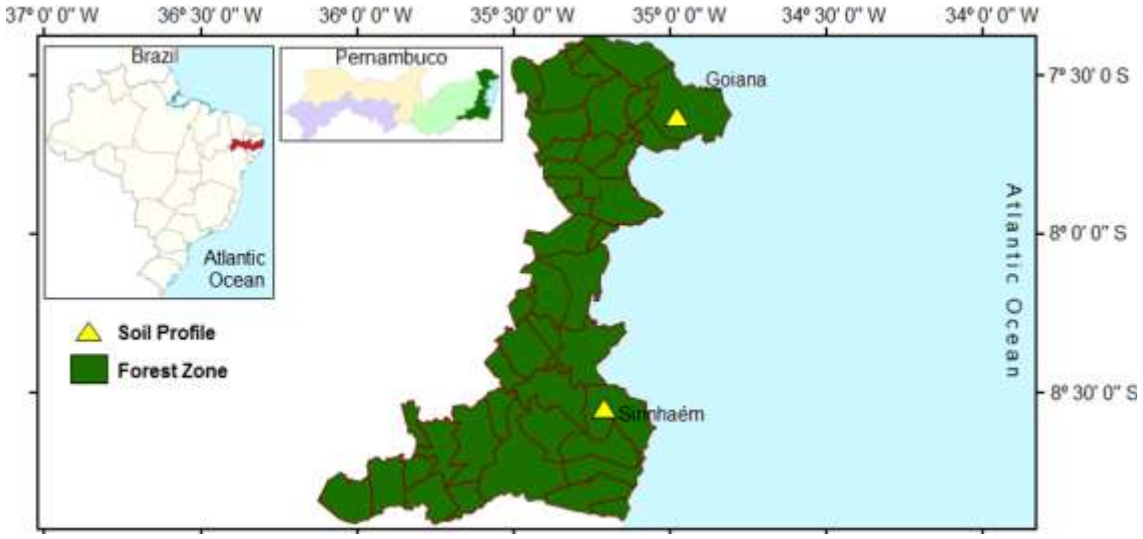


**Figure 1.** Location of soil profiles in the Forest Zone in Pernambuco State.

quality indicators can be selected according to the function of interest (Nortcliff, 2002). In recent decades, research has been conducted in order to establish quantitative parameter diagnostics of cohesion in soils, which broadens the discussion and provides their identification in agro-ecosystems (Silva et al., 2006; Silva et al., 2007). Thus, these parameters can act properly as change monitoring tools on the soil's physical quality at cohesive horizons under different management.

Studies involving water-soluble polymers used as chemical soil conditioners are concentrated on structural stability parameters and discussions about infiltration rates and sediment transport (Mamedov et al., 2009, 2010; Liu et al., 2009; Melo et al., 2014). Therefore, the absence of studies about soil structure is evident, with a focus on soil matrix qualitative arrangement and resulting pore space. Polyacrylamide (PAM) is one of the most important commercial polymers and has been widely used as a soil conditioner. PAM is made industrially with different molecular characteristics in terms of ionic type, molecular weight and charge density, reflecting its behavior in the solid-solution interface (Lentz and Sojka, 2009; Sepaskhah and Shahabizad, 2010). This study is aimed at evaluating the qualitative pore distribution and hydro-physical properties of cohesive and non-cohesive horizons of two soil profiles of Coastal Plains of Pernambuco State, Brazil, treated with polyacrylamide.

## MATERIALS AND METHODS

### Location and climatic characteristics of the study areas

Two soil profiles located at different regions in Pernambuco State, Brazil (Figure 1) were studied in 2012: (i) Goiana City, at the Experimental Station of Itapirema of the Agricultural Research Institute of Pernambuco - IPA (7° 37' 30'' S, 34° 57' 30'' W), with climate classified as Ams', according to Köppen, average annual rainfall 2,003 mm, vegetation predominantly sub-perennial rainforest; (ii) Sirinhaém City, (8° 36' 47'' S, 35° 19' 36'' W), with climate As' (Köppen) where the average annual rainfall is 1,310 mm and vegetation predominantly sub-perennial rainforest.

### Characterization and classification of soil profiles and selection of horizons

Soil profiles were classified as Ultisol (Goiana) and Oxisol (Sirinhaém) according to the Soil Taxonomy. Physical and chemical characterizations of the horizons are given in Table 1 and 2, respectively. The horizons for study were selected based on detailed morphological characteristics in the diagnosis of the cohesive character. Three horizons were selected in the Ultisol, horizon Bt1 (cohesive), and two non-cohesives (E and Bw/Bt horizons). From the Oxisol profile chosen for comparative cohesive character purposes under different pedogenetic conditions, the most characteristic cohesive horizon (BA) was selected, based on morphological characteristics. Disturbed samples were collected from the horizons for physical and chemical characterization.

### Chemical conditioner and sampling

The performance of the anionic polymer based on synthetic polyacrylamide (Polyacrylamide SuperflocA-130) at soil pore size distribution by diameter class and hydro-physical properties were evaluated in cohesive and non-cohesive soils. This polymer has a molecular weight of 15.0 Mg $mol^{-1}$ and charge density (hydrolysis) of 35%.

Undisturbed soil samples were collected in the field in block form (0.5 × 0.4 × 0.3 m), according to average thickness of horizons, and first wrapped in plastic film, then in bubble wrap, packed in styrofoam boxes to preserve their structure, and transported to Soil Physics Laboratory at Federal Rural University of Pernambuco (UFRPE). These blocks were placed in plastic trays and wrapped in a protective layer of gypsum of about 50.0 mm thickness, aiming at maintaining the block structure, which could be damaged by the pressure of collection when inserting the stainless steel cores (total volume ≅ 100 $cm^3$). After that, the blocks were moistened with

**Table 1.** Physical properties of the evaluated soil horizons.

| Horizon | Particle Size Analysis[1] | | | | | | Silt/Clay Ratio | DI[3] | FI[4] | Pd[5] | Bd[6] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total Sand | Coarse Sand | Fine Sand | Silt | Clay | WDC[2] | | | | | |
| | g kg$^{-1}$ | | | | | | | | | kg dm$^{-3}$ | |
| **Ultisol** | | | | | | | | | | | |
| E | 855.11 | 720.0 | 135.11 | 18.62 | 126.27 | 101.02 | 0.15 | 0.80 | 0.20 | 2.60 | 1.70 |
| Bt1 | 648.41 | 438.85 | 209.56 | 14.01 | 337.58 | 155.81 | 0.04 | 0.46 | 0.54 | 2.63 | 1.67 |
| Bw/Bt | 591.06 | 413.17 | 177.89 | 29.88 | 379.06 | 0.00 | 0.08 | 0.00 | 1.00 | 2.56 | 1.22 |
| **Oxisol** | | | | | | | | | | | |
| BA | 369.43 | 284.82 | 84.62 | 63.00 | 567.57 | 0.00 | 0.11 | 0.00 | 1.00 | 2.72 | 1.33 |

[1]Method of hydrometer reading with clay fraction after 24 h of settling (Almeida, 2008); [2]Water dispersible clay; [3]Dispersion Index = 1 - FI; [4]Flocculation Index = [(clay - water dispersed clay)/clay]; [5]Particle density: volumetric method pycnometry (Flint and Flint, 2002); [6]Bulk density: core method (Grossman and Reinsch, 2002).

**Table 2.** Chemical properties of the evaluated soil horizons.

| Horizon | pH ($H_2O$)[1] | $Na^+$ | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ | $Al^{3+}$ | H+Al | SB[2] | $CEC_{ef}$[3] | $CEC_{pot}$[4] | BS[5] | m[6] | ESP[7] | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | $mmol_c$ kg$^{-1}$ | | | | | % | | | mg kg$^{-1}$ |
| **Ultisol** | | | | | | | | | | | | | | |
| E | 4.9 | 0.0 | 0.1 | 10.6 | 0.3 | 2.8 | 12.5 | 11.1 | 13.9 | 23.6 | 47.1 | 20.2 | 0.0 | 5.1 |
| Bt1 | 4.9 | 0.1 | 0.1 | 11.3 | 0.7 | 3.1 | 14.5 | 12.3 | 15.5 | 26.8 | 46.0 | 20.3 | 0.6 | 3.1 |
| Bw/Bt | 5.0 | 0.1 | 0.0 | 6.3 | 0.5 | 3.5 | 17.0 | 6.9 | 10.4 | 23.9 | 29.0 | 33.5 | 0.4 | 9.0 |
| **Oxisol** | | | | | | | | | | | | | | |
| BA | 4.8 | 0.2 | 0.0 | 5.9 | 0.8 | 1.6 | 18.5 | 6.9 | 11.3 | 25.4 | 27.4 | 38.2 | 0.8 | 6.3 |

[1]pH in Water (1:2.5 soil to water ratio); [2]Sum of bases; [3]Effective cation exchange capacity; [4]Potential cation exchange capacity; [5]Bases saturation; [6]Aluminum saturation; [7]Exchangeable sodium percentage = ($Na^+$/$CEC_{pot}$) 100

distilled water by capillarity action to collect a soil core sample. Obtaining soil core samples were performed using a kind of sampler that inserts the core into the block continuously by the device hydraulics without impact. Thus, cores were inserted into soil blocks to obtain soil samples with the minimum possible disturbance.

### Treatments and application

Soil samples were treated with PAM aqueous solutions at three concentrations: 12.5; 50.0 and 100.0 mg kg$^{-1}$, and distilled water was used as a control treatment without PAM (0 mg kg$^{-1}$). The soil core samples were placed on plastic trays lined with foam (of thickness 20 mm) that was soaked with PAM aqueous solutions and distilled water as a control, to be taken up slowly by capillary action until saturation without change continuity of pores. After that, the core soil samples were removed from the trays and left to stand for 72 h: enough time to reach chemical equilibrium between the PAM solutions and soil matrix based on chemical kinetics of PAM adsorption (Deng et al., 2006; Melo et al., 2014). The experimental design was randomized blocks with four replications, so 16 soil core samples for each horizon were collected, totaling 64.

### Hydro-physical parameters

After standing time, the saturated hydraulic conductivity was given by the constant head permeameter method (Booltink and Bouma, 2002) and calculated according to the Darcy equation (Equation 1):

$$K_{sat} = \frac{Ve \times L}{A \times t \times (h+L)} \quad (1)$$

Where: $K_{sat}$ is hydraulic conductivity of the saturated soil (cm h$^{-1}$); Ve is effluent collected volume (cm$^3$); L is length of soil sample = 5 cm; A is cross-sectional area of the soil column = 5 cm$^2$; t is time (h); and h is hydraulic head = 1.7 cm. The total porosity of the horizons was quantified by the soil moisture saturation method; the water volume is equivalent to pore volume and is calculated as follows:

$$P = \frac{V_{pores}}{V_t} \quad (2)$$

Where: P is total porosity, m$^3$ m$^{-3}$; $V_{pores}$ is pores volume in m$^3$,obtained from difference between saturated soil mass and dry soil mass at 105°C, transforming water mass to volume (assuming water density =1,000 kg m$^{-3}$); $V_t$ is total volume, assumed to be equal to core volume ($10^{-4}$ m$^3$). The pore size distribution by diameter class was performed using sand table. Macroporosity was determined at the sand table by 1 kPa of soil suction and calculated as follows:

$$\text{Macroporosity} = \frac{V_{macropores}}{V_t} \quad (3)$$

Where: Macroporosity, m$^3$m$^{-3}$; $V_{macropores}$ is macropores volume,

**Table 3.** Hydraulic conductivity mean and standard deviation in saturated soil (Ksat) of horizons E, Bt1 and Bw/Bt (Ultisol) and BA (Oxisol) with the application of PAM aqueous solutions (12.5, 50.0 and 100.0 mg $kg^{-1}$) and distilled water (control = 0 mg $kg^{-1}$).

| Horizon | Aqueous Solution (mg $kg^{-1}$) | | | |
|---|---|---|---|---|
| | 0 | 12.5 | 50.0 | 100.0 |
| | Ksat (cm $h^{-1}$) | | | |
| **Ultisol** | | | | |
| E | 38.72 ± 11.47 | 43.99 ± 27.75 | 71.44 ± 40.52 | 44.63 ± 32.35 |
| CV[1] | 29.62 | 63.08 | 56.72 | 72.48 |
| Bt1 (cohesive) | 1.46 ± 0.98 | 0.51 ± 0.44 | 0.56 ± 0.20 | 0.62 ±0.20 |
| CV[1] | 67.28 | 87.19 | 36.29 | 40.65 |
| Bw/Bt | 7.35 ± 5.81 | 4.62 ± 4.77 | 21.38 ± 31.61 | 5.69 ± 4.59 |
| CV[1] | 79.01 | 103.15 | 147.82 | 80.75 |
| **Oxisol** | | | | |
| BA (cohesive) | 1.99 ±0.39 | 1.39 ± 0.88 | 1.08 ±0.53 | 1.46 ± 0.23 |
| CV[1] | 19.69 | 63.41 | 49.61 | 15.63 |

[1]Coefficient of variation (%).

obtained by water volume taken off the soil sample after reaching equilibrium at 1 kPa, $m^3$; $V_t$ is total volume, assumed to be equal to core volume ($10^{-4}$ $m^3$). From the data of the total porosity, the ratio macroporosity/total porosity (Macro/P) was calculated, as initially proposed by Taylor and Aschcroft (1972). Mesoporosity was determined at 6 kPa in the sand table, and calculated as follows:

$$\text{Mesoporosity} = \frac{V_{\text{mesopores}}}{V_t} \quad (4)$$

Where: Mesoporosity, $m^3m^{-3}$; $V_{mesopores}$ is mesopores volume, obtained by water volume taken off soil sample after reaching equilibrium at 1 and 6kPa, $m^3$; $V_t$ is total volume, assumed to be equal to the core volume ($10^{-4}$ $m^3$). Microporosity ($m^3$ $m^{-3}$) was quantified as total porosity minus macro and mesoporosity, according to Equation (5):

$$\text{Microporosity} = P - (\text{Macroporosity} + \text{Mesoporosity}) \quad (5)$$

The soil water retention curve (SWRC) was done in undisturbed samples at sand table (Romano et al., 2002) in the low-tension range (0 to 10 kPa), and the pressure plate extractor (Dane and Hopmans, 2002) was used to higher tensions (10 to 1500 kPa). At SWRC the measured matric potential ($\Psi$) was converted to soil water content ($\theta$) according to van Genuchten (1980), using the RetC software of Soil Salinity Laboratory (van Genuchten et al., 1991). Thus, we obtain the empirical parameters of fitting using Equation (6):

$$\theta = \theta_r + \frac{(\theta_s - \theta_r)}{[1+(\alpha\Psi)^n]^m} \quad (6)$$

Where, $\theta$ is soil water content, $cm^3$ $cm^{-3}$; $\theta_r$ is soil residual water content, corresponding to permanent wilting point, $cm^3$ $cm^{-3}$; $\theta_s$ is soil saturated water content, $cm^3$ $cm^{-3}$; $\Psi$ is soil water potential (cwc); $\alpha$ is a scale parameter inversely proportional to mean pore diameter, $cm^{-1}$; $n$ and $m$ are shape parameters of soil water retention curve, $m = 1 - 1/n$, $0 < m < 1$, according to van Genuchten (1980). Root penetration resistance (PR) was quantified in the soil core samples containing moisture equilibrated at 10 kPa, determined by an electronic penetrometer bench with a needle to simulate root penetration in the soil. The penetrometer operated at 1 cm $min^{-1}$ speed and the cone base was 4mm thick. The data acquisition system was connected to the penetrometer, and PR was expressed in MPa.

### Statistical analysis

The results were analyzed using descriptive statistics and subjected to analysis of variance (ANOVA), and the means compared by the Scott-Knott test ($p < 0.05$) using the statistical program SAEG (2009).

## RESULTS AND DISCUSSION

Among the soils treated with PAM aqueous solutions, only the Bw/Bt horizon at Ultisol showed no consistent behavior of hydro-physical properties. This horizon is intermediate between Bw and Bt horizons (Melo et al., 2014); thus it has materials of both horizons, which can explain such behavior.

In other horizons, PAM aqueous solutions provided changes in Ksat values influenced by both soil texture and solution viscosity. Ksat value increased in the E horizon, which is typically sandy soil, and in cohesive soils from horizons Bt1 and BA, both clay soils, there was reduction in permeability with application of PAM (Table 3).

The viscosity has a greater influence in soils where macropores are predominant, as evidenced by the reduction of Ksat in half on E horizon, when 100 mg $kg^{-1}$ was used compared to 50 mg $kg^{-1}$. A similar trend was found by Ajwa and Trout (2006), who obtained a reduction of Ksat in coarse soil when increasing the polymer concentration. According to them, the negative effects of PAM solution viscosity in infiltration rates are less harmful compared to hydraulic conductivity, due to

**Table 4**. Macro, meso and micropores volume at E, Bt1 and Bw/Bt (Ultisol) and BA (Oxisol) Horizons with PAM aqueous solutions (12.5, 50.0 and 100.0 mg $kg^{-1}$) and distilled water (control = 0 mg $kg^{-1}$).

| Soil | Horizon | Aqueous Solution (mg $kg^{-1}$) | | | |
|---|---|---|---|---|---|
| | | 0 | 12.5 | 50.0 | 100.0 |
| **Macropores ($cm^3$)** | | | | | |
| Ultisol | E | 3.55 ± 0.35 | 4.44 ± 0.58 | 4.27 ± 0.67 | 4.78 ± 0.64 |
| | CV[1] | 11.79 | 7.34 | 26.03 | 11.10 |
| | Bt1(cohesive) | 6.45 ± 1.68 | 3.62 ± 0.58 | 3.27 ± 0.23 | 3.61 ± 0.68 |
| | CV[1] | 25.89 | 16.60 | 7.21 | 19.14 |
| | Bw/Bt | 5.18 ± 1.08 | 4.11 ± 0.65 | 5.43 ± 1.98 | 4.82 ± 1.29 |
| | CV[1] | 20.85 | 15.77 | 36.23 | 26.64 |
| Oxisol | BA(cohesive) | 5.53 ± 0.68 | 3.56 ± 0.26 | 3.56 ± 0.92 | 3.69 ± 0.43 |
| | CV[1] | 11.79 | 7.34 | 26.03 | 11.10 |
| **Mesopores($cm^3$)** | | | | | |
| Ultisol | E | 17.39 ± 1.66 | 17.40 ± 1.79 | 18.32 ± 2.46 | 17.85 ± 1.38 |
| | CV[1] | 16.46 | 9.24 | 14.80 | 11.14 |
| | Bt1(cohesive) | 4.88 ± 0.32 | 4.12 ± 0.70 | 3.50 ± 0.32 | 3.51 ± 0.59 |
| | CV[1] | 6.73 | 16.41 | 8.55 | 17.28 |
| | Bw/Bt | 8.17 ± 1.75 | 7.29 ± 2.92 | 7.55 ± 2.91 | 7.90 ± 2.61 |
| | CV[1] | 21,61 | 39,56 | 35,56 | 32.47 |
| Oxisol | BA(cohesive) | 7.66 ± 1.25 | 8.25 ± 0.75 | 8.19 ± 1.16 | 6.66 ± 0.69 |
| | CV[1] | 16.46 | 9.24 | 14.80 | 11.14 |
| **Micropores($cm^3$)** | | | | | |
| Ultisol | E | 13.55 ± 1.26 | 13.41 ± 1.58 | 13.99 ± 1.74 | 12.94 ± 0.87 |
| | CV[1] | 1.32 | 1.56 | 1.39 | 2.54 |
| | Bt1(cohesive) | 23.21 ± 2.48 | 22.58 ± 0.56 | 22.28 ± 0.31 | 22.14 ± 0.45 |
| | CV[1] | 10.49 | 2.69 | 1.46 | 2.48 |
| | Bw/Bt | 30.42 ± 0.19 | 29.63 ± 0.90 | 28.43 ± 2.47 | 29.09 ± 0.96 |
| | CV[1] | 0.72 | 3.40 | 8.61 | 2.63 |
| Oxisol | BA(cohesive) | 34.23 ± 0.69 | 34.74 ± 0.87 | 34.79 ± 0.71 | 34.77 ± 1.15 |
| | CV[1] | 1.32 | 1.56 | 1.39 | 2.54 |

[1]Coefficient of variation (%)

complex relationship between conductivity, water content and soil matric potential. These findings also explain the results of Trout et al. (1995), where an increase of infiltration rates was observed with PAM application in soils of fine texture.

Soil permeability is measured by Ksat, and as a soil intrinsic property, represents water seepage through pore spaces. According to Sojka et al. (1998), the PAM effect on the soil permeability depends on several soil properties, mainly texture; if any sediment is entrained in the flow, on furrow irrigation, it is readily flocculated in the presence of PAM. As a result, infiltration rate is increased, mainly on finer textured soils. Dexter and Richard (2009) point out that more macropores do not necessarily imply increasing of soil permeability, since it must be connected. According to a qualitative study of pore space in each horizon, there was a predominance of micropores with decreasing sequence in terms of volume: micropores<mesopores<macropores, except for the E horizon, where mesopores are predominant (Table 4).

PAM effects on macropore volume were consistent with Ksat values. Once again, soil texture was the predominant factor, that is, there was an increase in the macroporosity of the E horizon and reduction in cohesive horizons Bt1 and BA (Figure 2a) compared to the control ($p < 0.05$). For both of them, there were no differences between polymer solutions. We believe that soil texture can influence PAM effects in macroporosity from a physical-chemical point of view. Cohesive horizons are clay and have greater electronegativity that can be increased by polymer solutions, since this kind of PAM has 35% of carboxylic groups, thus, increasing negative charge density in soil dispersion. As a result, macroporosity is reduced. In this context, Green et al. (2004) highlighted the repulsion arising from the interaction between PAM charge density and high-activity

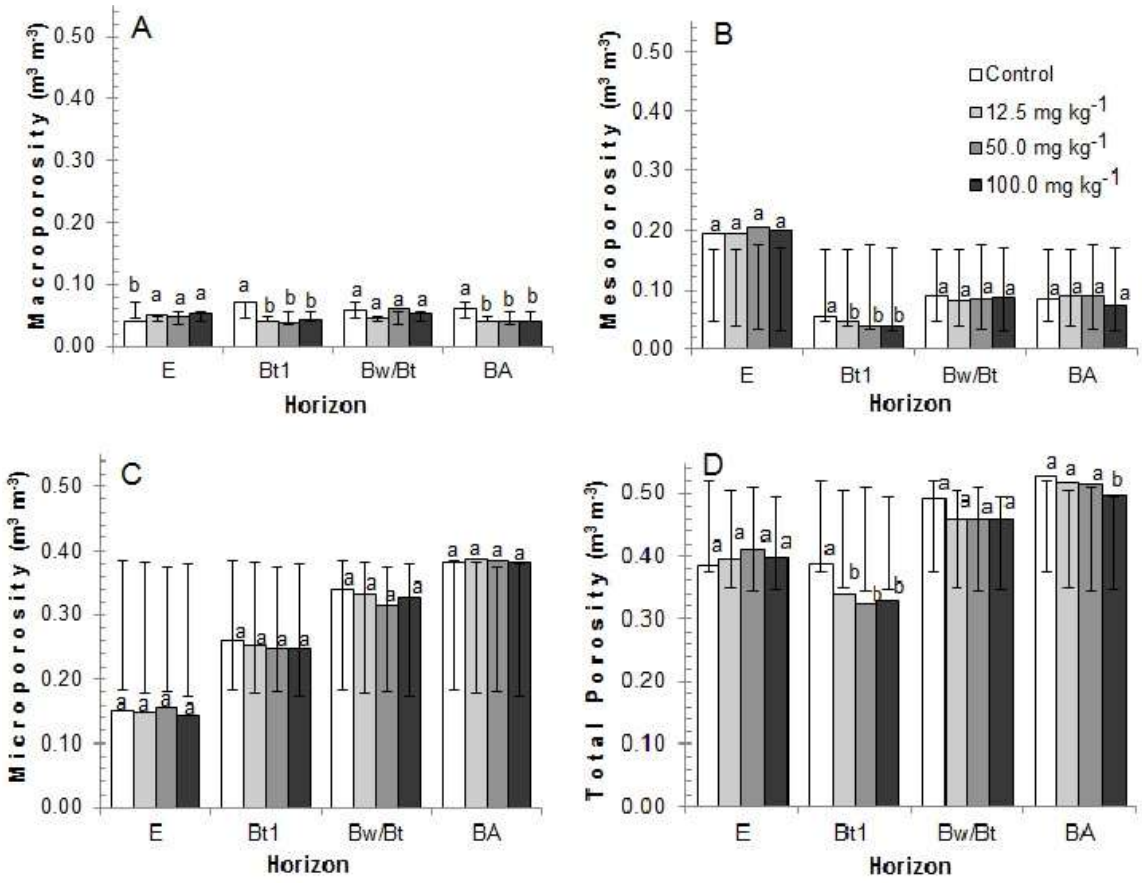


**Figure 2**. Macro, meso, microporosity and total porosity of E, Bt1, Bw/Bt (Ultisol) and BA (Oxisol) horizons with PAM aqueous solutions (12.5, 50.0 and 100.0 mg $kg^{-1}$) and distilled water (control=0 mg $kg^{-1}$). Means followed by the same letter with the same horizon = no significant difference using the Scott-Knott test ($p < 0.05$).

clays.

PAM solutions reduced the mesopores volume and consequently the mesoporosity of the Bt1 horizon (Table 4 and Figure 2b). On the other hand, there were no differences between treatments for microporosity ($p < 0.05$) (Figure 2c). Then, changes caused by PAM in the total porosity (P) at horizons were controlled mainly by macroporosity, with its reduction at cohesive horizons Bt1 and BA (Figure 2d). The most concentrated solution (100 mg $kg^{-1}$) provided the lowest values of total porosity at the BA horizon ($p < 0.05$). Lima et al. (2005) found total porosity at the cohesive horizon of Greying Ultisol as 0.34 $m^3$ $m^{-3}$, which was significantly lower than for non-cohesive horizons. This reduction occurred also for volume of macro and mesopores, with a predominance of micropores, similar to the Bt horizon here. The cohesive horizon BA presented the highest total porosity (Figure 2d) due to the higher micropores volume (Table 4 and Figure 2c). However, total porosity decreased at 100.0 mg $kg^{-1}$ PAM rate, due to the polymer effect on reduction of macroporosity (Figure 2a), since for mesoporosity and microporosity no significant differences were observed (Figures 2b and 2c, respectively). Corrêa et al. (2008) studied soils from three toposequences of Coastal Tablelands in Bahia and Espírito Santo (both states of Brazil) and found total porosity values for cohesive horizon (Bt1) ranging from 0.37 $m^3m^{-3}$ (Red Argisol - Ultisol) to 0.21 $m^3m^{-3}$ (Yellow Argisol - Ultisol). These values are lower than the cohesive soil of Coastal Tablelands of Pernambuco investigated here, which range from about 0.39 $m^3m^{-3}$ (Bt1) to 0.53 $m^3m^{-3}$ (BA) (Figure 2d). PAM aqueous solutions increased the PR values of Bt1 horizon (Table 5) similar to the study of Busscher et al. (2007), who used PAM doses of 30 and 120 mg $kg^{-1}$ in Acrisol. Strengthening the soil while increasing the volume would be consistent with the fact that PAM can improve aggregation, causing compact aggregates with larger inter-aggregate spaces; though this result could be unique to this study or condition.

The application form of PAM on soil may have affected our results of PR. Busscher et al. (2009) studied PR in two soils of US Coastal Tablelands, under field conditions, with different PAM concentrations, application forms and physical states (solution or granular), and concluded that treatments with the granular PAM had lower PR than those with the liquid PAM, probably a

**Table 5**. Soil penetration resistance of root system (PR) with soil moisture at 10 kPa in E, Bt1, Bw/Bt (Ultisol) and BA (Oxisol) horizons PAM aqueous solutions (12.5; 50.0 and 100.0 mg $kg^{-1}$) and distilled water (control=0 mg $kg^{-1}$).

| Horizon | Aqueous Solutions (mg $kg^{-1}$) | | | |
|---|---|---|---|---|
| | 0 | 12.5 | 50.0 | 100.0 |
| **PR (MPa)** | | | | |
| Ultisol | | | | |
| E | 0.60[a] | 0.86[a] | 0.60[a] | 0.91[a] |
| CV[1] | 23.62 | 27.04 | 30.62 | 45.24 |
| Bt1 (cohesive) | 1.20[c] | 2.52[b] | 3.46[a] | 3.16[a] |
| CV[1] | 37.24 | 16.19 | 13.71 | 15.41 |
| Bw/Bt | 0.32[a] | 5.34[a] | 5.76[a] | 4.82[a] |
| CV[1] | 105.11 | 99.36 | 87.40 | 66.47 |
| Oxisol | | | | |
| BA (cohesive) | 1.26[a] | 5.50[a] | 2.30[a] | 2.15[a] |
| CV[1] | 26.98 | 121.30 | 16.17 | 12.84 |

[1]Coefficient of variation (%); Means followed by the same letter with the same row = no significant difference using the Scott-Knott ($p < 0.05$).

result of being able to add higher amounts of PAM per ha in dry granular form. Thus, PAM can reduce penetration resistance by increasing soil aggregation, which disrupts the massive structure that constitutes the hard layer. Santana et al. (2006) defined a PR value of 2.0 MPa to characterize soils as cohesive, and highlighted how it is important to know the critical humidity below which cohesion manifests. Here, PR was determined on the samples under low matric potential (10 kPa), that is, moisture equivalent as a measure of the field capacity. Even so, we observed values above the critical value.

Macropores may be regarded as inter-aggregate pores (Othmer et al., 1991) and, therefore, as the PAM decreased macroporosity, this reduction was due to the reduction between the spaces of the aggregates, increasing soil aggregation, reflected by the PR increase in Bt1 compared to the control (Table 5). The PAM effect on macroporosity was evaluated also by Macro/P, which according to Taylor and Ashcroft (1972) must be 0.33 (dimensionless), considered an ideal value for crop development. In this study, two classifications of macropores size diameter (∅) were used to evaluate PAM influence on Macro/P ratio: (i) macropores with ∅ > 300μm and (ii) macropores with ∅ > 50μm. Both macropores size classifications resulted in a Macro/P ratio below 0.33 for cohesive soils, with the exception of the E horizon (Figure 3). In fact, the E horizon that has a sandy texture, basically has macropores, and when these were classified as pores with ∅ > 300 μm, the ratio values Macro/P were lower than 0.2 for all horizons, regardless of treatment (Figure 3b). However, when the macropores were ranked with ∅ > 50 μm, the ratio values Macro/P to E horizon were greater than 0.33 (Figure 3a). For other horizons, microporosity (Figure 2c) and macroporosity values (Figure 2a) explain the low values of the ratio Macro/P (Figure 3).

In cohesive horizons (Bt1 and BA), PAM application reduced the ratio Macro/P compared to the control (Figure 3), mainly for Bt1, where macro and mesoporosity were reduced (Figures 2a and 2b) due to the mechanism of action of PAM solutions reducing first the larger pores (inter-aggregates), as discussed in Akelah (2013). These results indicate that this kind of classification for macropores size of these soils could be re-evaluated using other criteria that can diagnose the resilience of them under treatment with chemical conditioners. Thus, we suggest that macropores could be better classified as pores of ∅ > 50μm. The macro/P relationship and pore size distribution of soil under treatment of chemical conditioners led to water movement in soil pores and consequently, water availability and retention in these pores. Soil size pore distribution influences the physical-hydraulic behavior. In this regard, soil water retention curves (SWRC) illustrate the soils' behavior under treatments (Figure 4).

Except for the Bw/Bt horizon, which showed a typical behavior in response to the action of PAM solutions (Figure 4c), the other horizons showed increased humidity from the field capacity ($\theta_{CC}$, when $\Psi \cong 10$ kPa $\cong$ 2.0 log cwc), for the concentration of 50.0 mg $kg^{-1}$compared to other treatments (Figure 4, red arrows). These results show that the action of PAM reduced the cohesion of these soils, mainly for the Bt1 and BA horizons in the drier range of SWRC, when cohesive soils become harder as the humidity decreased gradually, preventing the penetration of roots (Aly and Letey, 1989). In general, it is observed that soil water retention capacity was improved for the E horizon when PAM solutions

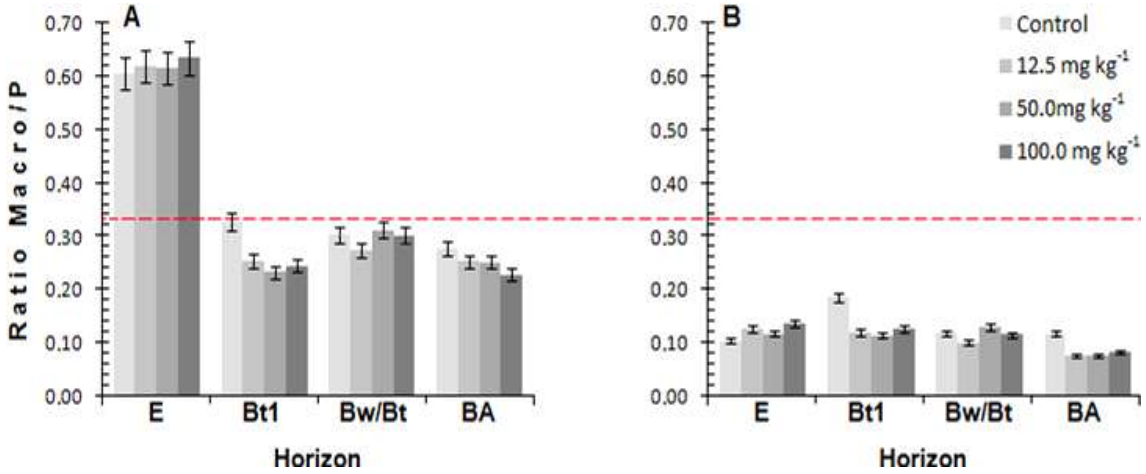


**Figure 3**. Macroporosity: total porosity (Macro/P) ratio at E, Bt1, Bw/Bt (Ultisol) and BA (Oxisol) horizons with PAM aqueous solutions (12.5, 50.0 and 100.0 mg $kg^{-1}$) and distilled water (control=0 mg $kg^{-1}$). Dashed line is dimensionless value of 0.33 as a reference: (A) macroporosity assumed macropores ∅ > 50 µm; (B) macroporosity assumed macropores ∅ > 300 µm.

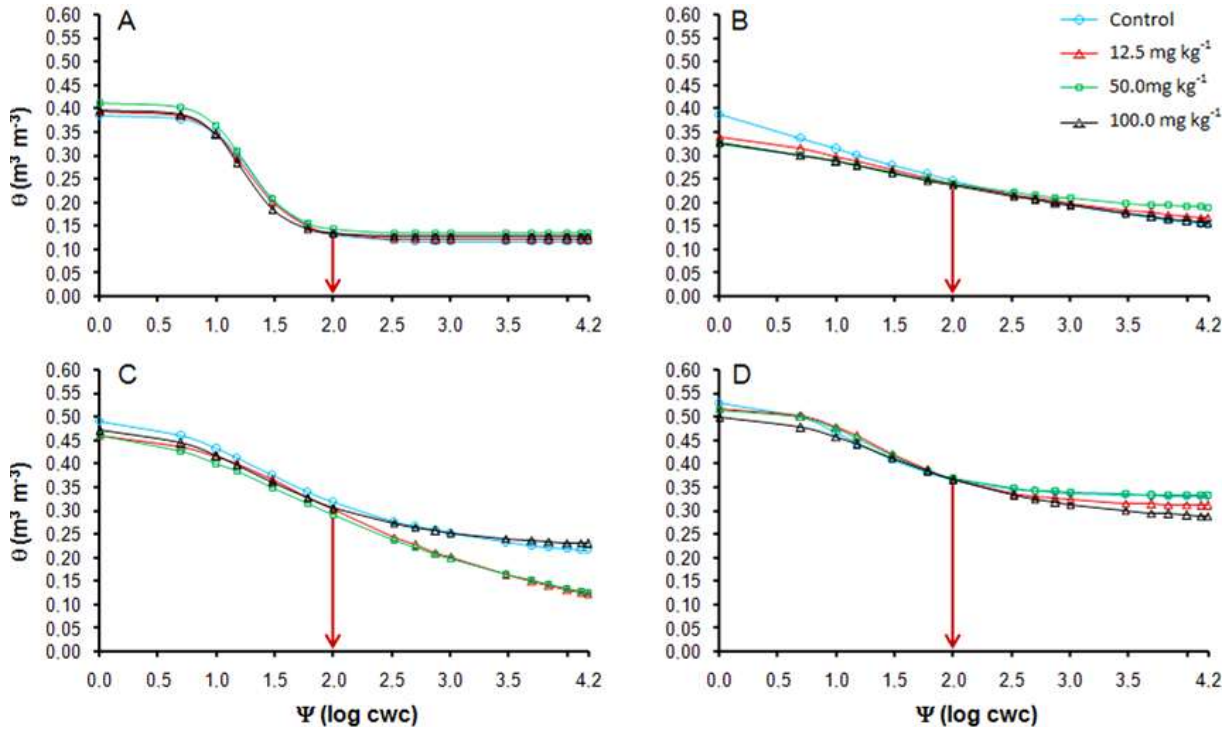


**Figure 4.** Soil water retention curve of E, Bt1, Bw/Bt (Ultisol) and BA (Oxisol) horizons with PAM aqueous solutions (12.5, 50.0 and 100.0 mg $kg^{-1}$) and distilled water (control = 0 mg $kg^{-1}$): (A) E Horizon; (B) Bt1 Horizon; (C) Bw/Bt Horizon; (D) BA Horizon, fitted according to the van Genuchten model by RetC software of Soil Salinity Laboratory (van Genuchten et al., 1991).

were applied, especially 50.0 mg $kg^{-1}$. Adding the amount of PAM in the sandy horizon (E) reduced the largest pores in the soils, and the pressure required for water expulsion is increased, as observed by Abedi-Koupai et al. (2008), when evaluated use of hydrogels, increasing the time that water will be available to plants. This result is very important, because this horizon is typically sandy with many macropores and loses water through seepage;

as a result, there is low water retention capacity (Figure 4a).

Abedi-Koupai et al. (2008) explain that volumetric water content increased due to the presence of a functional group (amide) on the chemical structures of PAM form hydrogen bonding with water. As a result, most of the water stored in the polymers is available to plants at relatively low tensions (Akelah, 2013). Regarding the effect of PAM at soil water retention capacity and water availability in cohesive horizons, SWRC of BA horizon (Figure 4d) compared to the Bt1 horizon (Figure 4b), is typical for soils with pore size distribution more assorted. Thus, we observed that the behavior of the BA curve (more winding) reflects higher values of meso and microporosity (Figures 2b and c), generally classified in structural pores (larger), and textural (smaller), as observed by Dexter and Richard (2009). On the other hand, the Bt1 curve (more horizontal), lower pitch is explained by higher values of microporosity (Figure 2c).These results are an indicator that cohesive horizon BA (Oxisol) has better structural conditions that favor soil physical-water properties.

Mamedov et al. (2009, 2010) also observed considerable effects on the shape of the SWRC for clay soils treated with PAM, which increased the water content, especially in the drier range of the curve. These authors explained that there was a possible effect of PAM in the hydration of aggregates, which increased their stability when wet (no slaking), reflecting the best hydro-physical conditions when the soil is dry.

Similarly, for cohesive horizons (Bt1 and BA), the effect of the application of PAM (50 mg $kg^{-1}$) can be observed at higher tensions (≥ 2.0 log cwc) than field capacity (Figure 4; after tensions indicated by red arrows).There is an increase in soil water storage when PAM was applied at 50 mg $kg^{-1}$, which indicates that this polymer can prevent or reduce water loss by seepage, as also verified by Lentz and Kincaid (2008).

## Conclusion

1. The PAM aqueous solution effect on Ksat of cohesive and non-cohesive soils depends both on soil texture and solution viscosity.
2. Except for the E horizon, micropores volume was prevalent, followed by mesopores and macropores.
3. Macroporosity on cohesive horizons was reduced by PAM solutions, which contributed to decreasing: Ksat, total porosity and Macro/P ratio, unlike for non-cohesive soils.
4. PAM solutions of 50 and 100 mg $kg^{-1}$ increased PR on the Bt1 cohesive horizon of Ultisol, distinct from other horizons.
5. The PAM solution effect on macroporosity redistribution of soil was more evident when the macropores were classified from ∅ > 50 µm.
6. PAM solutions with 50 and 100 mg $kg^{-1}$ provided a better distribution of pore sizes in cohesive soils, resulting in higher water retention in the high-tensions range of the SWRC.

## Conflicts of Interests

The authors have not declared any conflicts of interest.

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*Full Length Research Paper*

# Anthelmintic and antioxidant potential of *Fagopyrum esculentum* Moench *in vitro*

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**One of the limiting factors of sheep breeding is helminth infection, mainly resulting in poor weight gain. The combination of parasite control strategies, including the use of medicinal herbs can reduce the use of chemical anthelmintics. Tanniferous plants, by having phenolic compounds, mainly condensed tannins were associated with anthelmintic action. *Fagopyrum esculentum* Moench (buckwheat) has flavonoids, phenolic acids, tannins and high content of lysine, and the highest levels of these compounds are found in the seeds. Tannins exert direct anthelmintic action in reducing the the fertility of female nematodes, and indirect by increasing the immune response to protect the ingested protein of rumen degradation. The objective of this study was to evaluate the anthelmintic potential of the hydroalcoholic extract of *F. esculentum* Moench seeds (ETM) in the control of gastrointestinal nematodes of sheep *in vitro* and the antioxidant activity of ETM. Faeces from sheep presenting at least 2,000 eggs per gram of faeces were used. Hatchability and larvae migration tests were performed to evaluate the treatments ETM at concentrations of 0.625; 1.25; 2.5 and 5 mg $mL^{-1}$, negative and positive controls and DMSO control (0.75% + distilled water). The treatment means were compared by Tukey test at 5% of probability. Subsequently, the total content of polyphenols, flavonoids, tannins and antioxidant activity of ETM were determined. For the conditions evaluated in this study, it was possible to verify anthelmintic activity of ETM in both tests *in vitro*. The ETM inhibited 19.66% of hatching in concentration 1.25 mg $mL^{-1}$ and 17.66% of larvae migration in the concentration of 5 mg $mL^{-1}$. The anthelmintic activity may be due to condensed tannin content found (288.89 mg equivalent tannic acid per gram of extract). Antioxidant activity was observed at all concentrations, reaching 38.71% at 3 mg $mL^{-1}$ with $IC_{50\%}$ = 3.83 mg $mL^{-1}$ and 468.12 µM equivalent Trolox per gram of extract. At the same concentration to flavonoids and total polyphenols was observed respectively 31 mg equivalent rutin and 54.33 mg equivalent gallic acid per gram of extract. In addition to the direct effect of ETM on trichostrongylids of sheep, future research is also justified by the possibility of an indirect effect due to immune stimuli that protein diet provides on fostering in combating worms. It was concluded that it was possible to verify anthelmintic and antioxidant activity, demonstrating the potential of ETM in parasitological control of sheep.**

**Key words:** Agroecology, buckwheat, condensed tannins, gastrointestinal nematodes, organic production, sheep breeding.

## INTRODUCTION

Sheep breeding is an expanding activity (Güetter, 2011; Gianlorenço, 2013). Brazil is the 18th largest producer with herd estimated at 25.43 million of sheep and goats (De Zen et al., 2014). However, gastrointestinal nematodes are a major limiting factor to sheep production, especially in tropical regions (Vieira, 2008).

Damages caused by helminth infection include less weight gain, poor quality of wool, higher mortality, lower yield carcass, lower milk production and low fertility. These losses are due to clinical signs such as diarrhea, anemia, hemorrhage, prostration and weakness, adversely affecting profitability and animal welfare (Szpatowski, 2010). In addition, there are increased costs for the acquisition of antiparasitic drugs and labor use.

Among the parasites that infect sheep, are the trichostrongylid from Trichostrongylidae family, which includes species of genera *Trichostrongylus, Haemonchus, Ostertargia, Nematodirus* and *Cooperia*.

The integration of management systems is the main concept in the search for sustainable control of helminths (Hoste and Torres-Acosta, 2011). The search for strategies such as integrated grazing to other species and the use of natural therapies has been frequently observed (Batatinha et al, 2011; Joshi et al, 2011).

Another important aspect relates to the pharmacological resistance to chemical anthelmintics (Szpatowski, 2010). Natural measures for parasite control can minimize resistance to chemical anthelmintics (Houdijk et al., 2012). There are records of resistance to many drugs commonly used (Kaplan, 2004). Resistance has been observed in various chemical groups. The indiscriminate use of these drugs select resistant isolates, and therefore, these products do not end up performing the control of nematodes infections satisfactorily. In Brazil, the problem occurs in various regions (Vieira et al., 1992; Soccol and Pohl-de-Souza, 1997; Rosalinski-Moraes et al., 2007; Sczesny-Moraes et al., 2010, Vila Nova et al., 2014; Madruga et al., 2015). The resistance is gradually advancing on the latest available chemical groups. It is necessary to change the concept that the chemical anthelmintics are an inexhaustible source and only alternative for the control of parasites (FAO, 2003).

Today, the consumer market are showing growing demand for food products free from chemical residues (Resende, 2013). Organic farming, agroecology, biodynamic and organic have expanded rapidly in the world. These production systems contribute to the socio-economic sustainability of the producer (Neves et al., 2016). These systems are grounded in agroecological principles, not allowing the use of chemical pesticides.

Buckwheat (*Fagopyrum esculentum* Moench) is a dicotyledonous plant belonging to the Polygonaceae family, which has a high protein content, with high content of essential amino acid lysine (Zhou et al., 2012). The seeds have condensed tannins, flavonoids and phenolic acids (Steadman et al., 2001). Rutin, is a useful bioflavonoid in the treatment of various medical conditions, mainly due to its antioxidant action (Karamac, 2010). The highest concentration of rutin in buckwheat is found in the leaves and flowers (Vojtíšková et al., 2012).

Tannins exert direct anthelmintic action in reducing of the the fertility of female nematodes (Otero and Hidalgo, 2004), and indirect by increasing the immune response to protect the ingested protein of rumen degradation, increasing their availability in the lower gastrointestinal tract of animals (Ketzis et al., 2006). The anthelmintic activity *in vitro* of tannins was characterized by reduction of hatching, development and motility of larvae and adults (Brunet et al, 2008; Joshi et al 2011.). *In vivo* cause reduction of eggs per gram of faeces (OPG) and the parasite load (Minho et al, 2008; Max et al, 2009; Mupeyo et al, 2011; Oliveira et al, 2011).

Therefore, the objective of this study was to evaluate the anthelmintic potential of the hydroalcoholic extract of *Fagopyrum esculentum* Moench seeds (ETM) in control of gastrointestinal nematodes of sheep *in vitro*, as well as to evaluate the antioxidant activity, total content of polyphenols, flavonoids and condensed tannins.

## MATERIALS AND METHODS

### Plant material and extract preparation

Buckwheat seeds were collected in commercial farming in São João do Ivaí, Paraná, Brazil. The botanical material was selected by the absence of macroscopic changes in its surface constitution. Drying was carried out in a forced ventilation air oven at a temperature of 40°C and then maceration in a Wiley mill.

To obtain the hydroalcoholic extract of buckwheat seeds to 10%, 90 g of seed, 240 mL of distilled water and 570 mL of absolute ethanol were used. After this, the solution was kept under constant mechanical stirring at environment temperature for 24 h. Subsequent vacuum filtration was performed three times, after each filtration adding new hydroalcoholic solution in the same above ratios. The extract was concentrated in rotaevaporator and lyophilized.

### Obtainment of eggs and larvae of nematodes

Faeces were collected directly from the rectum of naturally infected sheep and free from previous chemical treatments at least 60 days. Eggs per gram of faeces (EPG) was performed for selection of

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animals with EPG value above 2,000 eggs.

For eggs isolation for hatchability test, the methodology described by Coles et al. (1992) adapted by Bizimenyera et al. (2006) was used. Faeces were macerated in water at 40°C, sieved through sieves of 250, 150, 75 and 25 µm. After the material was centrifuged at 3000 revolutions for 5 min, the supernatant was discarded. This material was then transferred to Falcon tubes, filled with saturated NaCl solution suspending the eggs. After further centrifugation, the supernatant was again filtered on sieve of 25 µm and washed under the conditions described above. To obtain larvae coproculture was performed (Ueno and Gonçalves, 1998).

**Anthelmintic evaluation**

The hatchability test was carried out in quadruplicate (Von Samson-Himmelstjerna et al., 2009), evaluating the following treatments: ETM at concentrations of 0.625; 1.25; 2.5 and 5 mg $mL^{-1}$, negative control (distilled water), positive control (albenzadol sulfoxide 0.25 mg $mL^{-1}$ associated with dimethylsulfoxide - DMSO 0.75%) and DMSO control (distilled water with 0.75% DMSO).

To evaluate each treatment, 100 µL of the suspension water and eggs (with 110 eggs), and 400 µL of the respective treatments was added to the culture plates. The plates were incubated in biochemical oxygen demand oven (B.O.D.) at 27°C for 48 h. After, total count of eggs and first stage larvae ($L_1$), was performed to obtain the hatchability percentage.

To perform the inhibition of larval migration test (Rabel et al., 1994) aqueous solution containing 150 third-stage larvae ($L_3$) obtained by coproculture in 100 µL of solution was standardized. They were evaluated in quadruplicate, the same aforementioned treatments, except for the positive control treatment, which consisted of 0.01 mg $mL^{-1}$ levamisole hydrochloride associated with 0.75% DMSO.

Microtubes containing 1 mL of the respective treatment solution and 100 µL of $L_3$ were incubated in B.O.D. at 37°C for two hours, then centrifuged at 6000 revolutions per 3 min. 900 µL was removed and the volume remained as 200 µL.

For test preparation, 24 well culture plates were used, each with a filter opening of 25 µm. In each filter, 1800 µL of each treatment and the remaining 200 µL of the respective wells for each treatment were added.

Plates were again incubated in B.O.D. for two hours at 37°C and after the filters were removed for counting larvae migrated and were retained. Larvae were inactivated with Lugol 5% and the reading was conducted under an optical microscope with 40x magnification.

The migration percentage was calculated by the formula % migration = [Nm / (Nm + Nr)] x 100, where Nm is the number of $L_3$ larvae migrate through the mesh and Nr is the number of larvae $L_3$ retained in the mesh. In both tests, the averages were compared by Tukey test at 5% probability by the Statistica software (Stat Soft, 2007).

**Determination of total polyphenols content, flavonoids and tannins of ETM**

The ETM was diluted in the concentrations 0.25; 0.50; 1; 1.5; 2 and 3 mg $mL^{-1}$ and evaluated in triplicate. For determination of total polyphenols, the method used was the Folin-Ciocalteu. The results were expressed in milligrams of gallic acid per gram of extract. Gallic acid is a precursor of several types of phenolic compounds has simple structure, which is considered standard substance (Stagos et al., 2012).

The dosage of total flavonoids was determined by UV-Vis spectrophotometer according to the methodology of Zhishen et al. (1999), based on the complexation of flavonoids with $AlCl_3$. The results were expressed in milligrams of rutin per gram of extract. The rutin and quercetin, shows the basic structure of flavonoids and can be used as an indirect indicator of flavonoids.

The determination of the content of tannins was according to the methodology of Makkar (1994) adapted by Fagbemi et al. (2005), and the results were expressed in milligrams of tannic acid per gram of dry extract.

**Determination of antioxidant activity of ETM**

The antioxidant activity of the extract was determined by the ability of $H^+$ donor for the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), according to Blios (1958). The calculation of the antioxidant activity was performed according to the formula: Antioxidant activity (%) = $[(A_{control} - A_{sample}) / A_{control}] \times 100$, where $A_{sample}$ is the absorbance of the samples after 30 min and $A_{control}$ is the absorbance of DPPH; both at 517 nm.

Subsequently, the antioxidant activity was also determined by the iron reduction method (FRAP test) according to Rufino et al. (2006) using solution Trolox 1000 µM as standard. Results were expressed in µM Trolox equivalent per gram of dry extract.

## RESULTS AND DISCUSSION

Through the EPG, trichostrongylids eggs were identified. It was possible to verify anthelmintic activity of ETM in inhibiting hatching and larval migration. The results corroborated with other researchers that reported inhibitory action of tanniferous plants on hatchability and migration larvae of nematodes in sheep (Bizimenyera et al., 2006; Maciel et al., 2006; Yoshihara et al., 2014) (Table 1).

For positive controls, albendazole sulfoxide 0.25 mg $mL^{-1}$ showed 100% inhibition of hatching and levamisole hydrochloride 0.01 mg $mL^{-1}$ showed 89% inhibition of larval migration. The control DMSO did not differ ($p<0.05$) from the negative control in hatchability test, but differed in migration test, showing inhibition of 9.60% in hatchability test and 7.14% in migration test.

The anthelmintic activity presented by ETM, probably was due to the condensed tannin content, which in the concentration 3 mg $mL^{-1}$ reached 288.89 mg tannic acid equivalent per gram of extract (Table 2). However, higher concentrations of ETM (> 2.5 mg $mL^{-1}$) did not differ from NC and DMSO in hatchability test. Thus, these results represent an important perspective for control of nematodes by the consumption of tanniferous plants.

Bizimenyera et al. (2006) reported a 100% inhibition of hatching of larvae of *Trichostrongylus colubriformis* with 25 mg $mL^{-1}$ of extracts from different parts of *Beltophorum africanum*. Maciel et al. (2006) worked with the same plant at the same concentration and found that the ethanolic extract of the leaves inhibited 100% hatching larvae of *Haemonchus contortus*, while the hexane extract at the concentration 50 mg $mL^{-1}$ inhibited only 16.92% of hatching. According to the authors, the mechanism of the anthelmintic action still had to be determined. However, it could be due to tannins, since the extracts with tannins removed exhibited slightly less activity than the crude extracts.

**Table 1.** Arithmetical average of hatching and migration of third-stage larvae inhibition of gastrointestinal nematodes of sheep percentage and their standard deviation in treatment.

| Treatment (mg mL$^{-1}$) | Hatchability inibition (%)* | Larval migration inibition (%)* |
|---|---|---|
| ETM 0.625 | 11.69$^{b}$ ± 2.85 | 11.39$^{d}$ ± 1.19 |
| ETM 1.25 | 19.66$^{b}$ ± 0.45 | 11.63$^{cd}$ ± 1.76 |
| ETM 2.5 | 15.63$^{bc}$ ± 5.44 | 17.04$^{bc}$ ± 0.75 |
| ETM 5 | 8.65$^{c}$ ± 1.24 | 17.66$^{b}$ ± 1.35 |
| NC – Distilled water | 5.37$^{c}$ ± 0.75 | 4.81$^{c}$ ± 0.40 |
| DMSO Control | 5.27$^{c}$ ± 3.04 | 6.53$^{de}$ ± 1.03 |
| PC – Albendazol sulfoxide | 100$^{a}$ ± 0 | NT |
| PC – Levamisole hydrochloride | NT | 88.93$^{a}$ ± 4.29 |

ETM: Hydroalcoholic extract of buckwheat at 10%; NC: negative control; DMSO control: distilled water with 0.75% DMSO; PC: positive control; NT: not tested; *Means followed by the same letter in the column do not differ significantly by Tukey test at 5% of probability.

**Table 2.** Mean values for the total polyphenols, condensed tannins and flavonoids for different concentrations in mg mL$^{-1}$ of hydroalcoholic buckwheat extract at 10% (ETM).

| Concentration ETM (mg mL$^{-1}$) | Polyphenols (mg equiv. gallic acid)* | Tannins (mg equiv. tannic acid)* | Flavonoids (mg equiv. rutin)* |
|---|---|---|---|
| 0.25 | 19.18$^{c}$ ± 4.70 | ** | 0.57$^{d}$ ± 0.00 |
| 0.5 | 38.31$^{b}$ ± 1.54 | 17.23$^{e}$ ± 3.56 | 13.62$^{c}$ ± 4.36 |
| 1 | 53.00$^{a}$ ± 1.33 | 70.44$^{d}$ ± 7.05 | 19.19$^{bc}$ ± 2.18 |
| 1.5 | 57.04$^{a}$ ± 1.07 | 126.15$^{c}$ ± 12.79 | 22.95$^{b}$ ± 0.95 |
| 2 | 50.99$^{a}$ ± 3.13 | 176.15$^{b}$ ± 13.12 | 25.07$^{ab}$ ± 0.71 |
| 3 | 54.33$^{a}$ ± 0.68 | 288.89$^{a}$ ± 10.21 | 31.00$^{a}$ ± 1.72 |
| CV (%) | 30.18 | 71.37 | 54.16 |

*Per gram of extract. Means followed by the same letter in the column do not differ significantly by Tukey test at 5% of probability. **Value out of the standard curve. CV: coefficient of variation.

**Table 3.** Mean values for the antioxidant activity of ETM by the methods of the donor capacity of H+ to the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and reduced iron (FRAP test).

| Concentration ETM (mg mL$^{-1}$) | Antioxidant activity (AA%)* | Antioxidant activity (µM equiv. Trolox p/ g of ETM - FRAP)* |
|---|---|---|
| 1.5 | 21.59$^{c}$ ± 0.59 | 452.14$^{a}$ ± 41.28 |
| 2 | 27.22$^{b}$ ± 0.05 | 467.05$^{a}$ ± 17.07 |
| 3 | 38.71$^{a}$ ± 1.06 | 468.12$^{a}$ ± 31.70 |
| CV (%) | 26.00 | 6.15 |

*Means followed by the same letter in the column do not differ significantly by Tukey test at 5% of probability. CV: coefficient of variation.

Alonso-Diaz et al. (2008) found that the four tanniferous plant extract (*Acacia pennatula, Lysiloma latisiliquum, Piscidia piscipula* and *Leucaena leucocephala*) in the concentrations 1.2 mg mL$^{-1}$ inhibited 49.1 to 63.8% migration of larvae $L_3$ of *H. contortus*. All four plant extracts interfered with the process of L3 exsheathment, which might be involved as a mechanism of action of tannins on *H. contortus* larvae. Yoshihara et al. (2014) required 100 mg mL$^{-1}$ of a commercial extract of *Acacia mearnsii* for 97.1% inhibition of larval migration. In addition to the anthelmintic action, the extract caused ultrastructural changes in adult parasites *H. contortus* after contact *in vitro* and *in vivo* (Yoshihara et al., 2015). For flavonoids and tannins, the highest levels were found in the concentration of 3 mg mL$^{-1}$. For polyphenols, concentrations with higher levels were 1; 1.5; 2 and 3 mg mL$^{-1}$, which did not differ (Table 2).

ETM showed antioxidant activity (AA%) at all tested concentrations, reaching 38.71% at the concentration of 3 mg mL$^{-1}$ (Table 3). The $IC_{50\%}$ corresponds to 3.83 mg

$mL^{-1}$. For iron reduction test (FRAP), ETM reached 468.12 µM Trolox equivalent per gram of extract at the concentration of 3 mg $mL^{-1}$.

According to Sun and Ho (2005), buckwheat presents an effective antioxidant activity as compared to the natural antioxidants, and natural antioxidants may have the potential to prevent lipid oxidation of food. Karamac (2010) found that the antioxidant activity of tannins fraction of phenolic compounds from buckwheat showed $IC_{50\%}$ corresponding to 0.019 mg $mL^{-1}$.

The author concluded that comparing the antioxidant activity of tannin fractions from buckwheat with the literature data concerning the antioxidant activity of fractions isolated from other plants, leads to the conclusion that buckwheat fractions are strong antioxidants. The highest values found by Karamac (2010) for the antioxidant activity of buckwheat in relation to this study were due to the different way of extraction. The author obtained the antioxidant activity of isolated fractions of tannins (from seeds and groats), whereas the present study showed the hydroalcoholic extract of ground seeds.

The primary antioxidants in the buckwheat are rutin, quercetin and hyperin, and the bran and husk have from 2 to 7 times more antioxidant activity than grasses such as oats, tricicale and barley (Morishita et al., 2007; Holasova et al., 2002; Zdunczyk et al., 2006). The antioxidant activity of methanol extracts to 80% in different cultures presented the following order, considering the highest to lowest: buckwheat, barley, oats, wheat, rye (Zielinski and Kozlowska, 2000). Considering the antioxidants in buckwheat, there may be benefits in using this crop in animal feed due to the fact that it reduces the production of free radicals and can help prevent lipid oxidation, which increases the shelf life of the meat (Lima Júnior et al., 2013).

Inglett et al. (2010 and 2011) evaluated antioxidant activity in buckwheat extracts with water, hydroalcoholic solution 50% ethanol and 100% ethanol, using microwave irradiation or water bath for 15 min at different temperatures (23 to 150°C). Regardless of the heat source, higher antioxidant activities was found in 100% ethanol extract at 100 and 150°C: 5.61 and 5.73 µmol Trolox equivalent per gram of extract, respectively.

Rutin is a useful bioflavonoid in the treatment of various medical conditions, mainly due to its antioxidant action (Karamac, 2010). Flavonoids may have antimicrobial activity due to the disruption and destruction of microbial membranes by forming complexes of the bacterial wall with soluble proteins (Soldera et al., 2010). This represents additional advantage considering the possibility of the occurrence of secondary bacterial infections to gastrointestinal parasites. Likewise, the antioxidant activity of flavonoids is important due to the control of the production of free radicals (Sousa et al., 2007). The highest concentration of rutin in buckwheat is found in the leaves and flowers (Vojtíšková et al., 2012).

In addition to direct anthelmintic action that ETM presents, buckwheat in animal feed can still provide indirect action due to immune stimuli that protein diet provide favoring combating of worms. Protein supplementation determines reduction of eggs per gram of faeces values (Veloso et al., 2004; Igarashi et al., 2013).

At 51 days after sowing, Klein et al. (2010) found crude protein contents (CP) in a late and early cultivar of buckwheat to be, respectively 17.27% and 15%. These values are superior to commonly used fodder grasses. Italiano and Neto (2006) found 11.53% of CP in Andropogon, 10.58% in Tanzania and 9.15% in Tifton-85. Gorgen (2013) found in a buckwheat cultivar, crude protein levels at 47 and 57 days respectively, with 23.8 and 14.7%. Alencastro (2014) found in the cultivars tested at 50 and 70 days, 12.73 and 11.13% CP, respectively. The lower level is due to the fact that the advance of maturity of buckwheat causes reduction in crude protein, and the highest levels are observed in the vegetative stage, reducing after flowering. The result of the analysis of buckwheat forage is promising for animal feed, mainly for dairy cattle in the fall season with problem of lack of forage (Klein et al., 2010).

Additionally, buckwheat has a high content of lysine, an essential amino acid deficient in most cereals, demonstrating potential in animal feed (Joshi and Padora, 1991; Kunachowicz et al., 1996). Buckwheat can also act as functional forage manipulating ruminal fermentation and providing reduction in methane formation by 12% (Amelchanka et al., 2010; Leiber et al., 2012).

## Conclusion

For the conditions evaluated in this study, it is concluded that anthelmintic and antioxidant activity, demonstrate the potential of ETM in parasitological control of sheep.

## Conflict of Interests

The authors have not declared any conflict of interests.

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# Productivity and quality of watermelon as function of phosphorus doses and variety

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**Although watermelon is one of the major vegetable-fruit crop, management fertilization still lacks information for increased productivity, quality and profitability, and to lower environmental impact. An experiment was conducted in Mossoró, Brasilian city of Rio Grande do Norte, from August to October 2012, to evaluate the effects of doses of phosphorus (P) (0, 45, 90, 135, 180 and 225 kg ha$^{-1}$ $P_2O_5$) in productivity, quality and profitability of two cultivars of watermelon cultivation (Top Gun and Olympia), in a randomized blocks in a factorial 6 × 2, with four replications. Larger number of commercial fruits (1.70 per plant) and commercial yield (74.39 t ha$^{-1}$) were obtained with 54.8 and 49.4 kg ha$^{-1}$ $P_2O_5$, respectively. Attributes of quality of watermelon fruit were not influenced by P. Dose between cultivars differences were found, and 'Olympia' had higher fruit mass and skin thickness than the 'Top Gun'. However, the soluble solids content of the 'Top Gun' was 4.9% higher than the Olympia. The maximum economic return was achieved with a dose of 49.37 kg ha$^{-1}$ of $P_2O_5$.**

**Key words:** *Citrullus lanatus*, phosphate fertilizer, production cost.

## INTRODUCTION

Watermelon (*Citrullus lanatus* L.) has stood out among the main horticultural products in Brazil. In the past years, there has been an increase in the area planted with the crop, especially in the brazilian states from the north and northeast. From 2001 to 2014, the area planted with watermelon in Rio Grande do Norte had a 310% increase, going from 1,655 to 5,133 hectares, while in the same period there was 13% reduction in the productivity

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**Table 1.** Physical and chemical characteristics of the soil to a depth 0-20 cm, sampled prior to the experiment.

| Parameter | Values |
|---|---|
| pH (water) | 5.9 |
| P (mg $dm^{-3}$) | 6.4 |
| K ($cmol_c$ $dm^{-3}$) | 0.16 |
| Na ($Cmol_c$ $dm^{-3}$) | 0.02 |
| Ca ($Cmol_c$ $dm^{-3}$) | 1.21 |
| Mg ($Cmol_c$ $dm^{-3}$) | 0.11 |
| Al ($Cmol_c$ $dm^{-3}$) | 0.05 |
| H + Al ($Cmol_c$ $dm^{-3}$) | 3.22 |
| OM (%) | 0.24 |
| CEC ($Cmol_c$ $dm^{-3}$) | 4.72 |
| V (%) | 32 |
| sand (g $kg^{-1}$) | 820 |
| silt (g $kg^{-1}$) | 40 |
| clay (g $kg^{-1}$) | 140 |

(0.0125 mol $L^{-1}$ H2SO4 and 0.050 mol $L^{-1}$ HCl at a soil: extractant 1:10); Ca, Mg and Al = (KCl 1 mol $L^{-1}$ in soil: 1:10 extractor); (H + Al) = (calcium acetate 0.5 mol $L^{-1}$ in soil: 1:15 extractor); sand, silt and clay = pipette method; CEC = cation exchange capacity; OM = organic matter; V = base saturation.

(PAM, 2014). Along with the increase in the producers' interests for growing watermelon, there is also growth in the use of watermelon hybrids, mainly due to its productive potential. However, productivity has declined in recent years (PAM, 2014), demonstrating that this is a characteristic of low heritability (Ferreira et al., 2003) and strong environmental influence, it becomes then necessary to review the management currently adopted for this crop. Among the several production factors, fertilization is one of the factors that most lack information in the watermelon crop. Many producers, despite using hybrids, use old information for crop fertilization (Trani et al., 1997; CFSEMG, 1999; Cavalcanti et al., 2008), based on cultivars of open pollination and smaller productive potentials and, consequently, demand of nutrients. Among the nutrients, we can highlight phosphorus (P), which, despite being absorbed in small amounts by the watermelon plant (Grangeiro and Cecílio Filho, 2004; Nogueira et al., 2014), is key to obtain high productivity and quality of watermelon fruits. P is a component of nucleic acids (e.g. DNA), phospholipids, enzymes and molecules such as ATP (where the plant stores metabolic energy); it is present in the structures of the cellular membrane and participates in the synthesis of proteins and vitamins. Phosphorus also has an important function in the energy transfer system inside the plant, taking part in processes such as photosynthesis and respiration (Hawkesford et al., 2012). The use of this nutrient in the appropriate dose and time, favors growth of the roots, flowering and fruit set, besides accelerating their ripening and improving the sugar content (Molina, 2006). Most Brazilian soils (91%) have a low content (<9 mg $dm^{-3}$) or middle (9 to 12 mg $dm^{-3}$) P (Lopes, 1998; Anghinoni, 2004) then being considered phosphorus poor, owing to their source material and high determination of this element, causing low P content available, especially in soils where there is a predominance of minerals sesquioxides (Novais and Smyth, 1999; Raij, 2011), for which there is often shortage of element in plants resulting in low productivity (Leon et al., 2008; Maluki et al., 2015.). However, excess phosphorus fertilization can also be harmful to the plant, especially the antagonistic effect on zinc absorption (Souza et al., 2011a), as well as to the environment due to eutrophication of water sources (Bennett et al., 2001). The present paper aimed at assessing the effect of doses of phosphorus upon productivity, quality and profitability of the watermelon crop.

## MATERIALS AND METHODS

### Site location and characterization

The experiment was done from August to October 2012, in Mossoró, RN, with geographic coordinates 5° 03' 37" S and 37° 23' 50" W and altitude of 72 meters. During the experiment period, the average temperature and the average relative humidity were 26.7°C and 56.8%, respectively, without rain. The soil of the experimental area was classified as Red Yellow Argisol (EMBRAPA, 2006), whose physico-chemical characteristics of the layer 0-20 cm are presented as in Table 1.

### Treatments and experimental design

The experimental design was randomized complete blocks, with four replications. The treatments were arranged in a 6 × 2 factorial design, composed by six doses of phosphorus (0, 45, 90, 135, 180 and 225 kg $ha^{-1}$ of $P_2O_5$) and two watermelon (*Citrullus lanatus*) cultivars ('Top Gun' and 'Olímpia'). Each experimental unit was composed by three rows of eight plants, with a space of 2.5 m between the rows and 0.8 m among the plants, the total area was 48 $m^2$, and the plants used were the eight plants from the central row from each parcel as useful area.

### Field establishment

Soil preparation consisted of plowing and harrowing, followed by thrown distribution of limestone (Relative Neutralization Total Power of 95%), in total area, and incorporation with grid, in order to elevate base saturation to 70%. Irrigations by sprinklers were done for 20 days, using a 5 mm daily slide. After this period, the next step was furrowing, with approximately 10 cm deep, and fertilization of planting with triple superphosphate (43% $P_2O_5$), in the dose corresponding to each treatment. Right after that, the sites were raised and drip irrigation was employed, with one hose per site, with issuers with 0.40 m in space between each other and 1.5 L $h^{-1}$ of flow. The sites were covered with black plastic, and holes of approximately 4 cm in diameter were made in it, with a 0.80 m space between each other. The seedlings were produced in polyethylene trays with 200 cells, and transplanted when they presented one permanent leaf. After that, the plants from each site were covered with white polypropylene tissue, weighting 15 g $m^{-2}$,

forming a tunnel in order to avoid the attack of leafminer (*Liriomyza trifolli*) and the whitefly (*Bemisia tabaci*), so the cover remains for 15 days. Topdressing was done daily through irrigation water, beginning right after transplanting, and the total following amounts were applied 98.63; 198.53; 32.88; 6.26; 2.04 and 7.8 kg $ha^{-1}$ of N, $K_2O$, Ca, Mg, B and Zn, respectively. The fertilizers used were urea, potassium nitrate, potassium chloride, calcium nitrate, magnesium sulfate, boric acid and zinc sulfate and 1.16 L $ha^{-1}$ of Root® (amino acid based product used to improve rooting of seedlings). Irrigation was done daily according to the need of the crop, with a total slide in the cycle of 286.4 mm.

**Harvesting and parameters evaluated**

The harvests of fruits were done in the useful area of the experimental unit, at 56 and 63 days after transplantation, and an evaluation was made of:

a) Total and commercial productivity (t $ha^{-1}$): total productivity corresponded to the sum of the masses of healthy fruits (from all classes), while for the commercial productivity (t $ha^{-1}$) fruits with mass ≥ 5.0 kg were considered;
b) Number of fruits per plant, total and commercial (fruits $plant^{-1}$): obtained by dividing the number of total or commercial fruits by the number of plants;
c) Mass of the fruits and commercial fruits (kg): corresponded to the result of the division of total or commercial productivity by the number of total or commercial fruits, in 1 hectare;
d) Content of solid soluble (ºBrix): portions of the pulp were removed from close to the flower scar, central and close to the fruit peduncle, which were homogenized by extracting juice, and the readings were determined in a digital refractometer;
e) Titratable acidity (g citric acid per 100 mL of juice): at 1 mL of the pulp juice, used to evaluate the content of soluble solids, 49 mL of distilled water and 2 drops of phenolphthalein 1% were added. Titration was done until the turning point with NaOH solution (0.1 N), previously standardized;
f) Peel thickness (mm): determined with the employment of caliper rule in the median region of the fruit; Pulp firmness (N): obtained by the average of three readings in the pulp, one in the distal end, one in the center, and another close to the peduncle, with the help of a manual penetrometer with an 8 mm diameter door;
g) Content of phosphorus on the leaf: was removed from the fifth leaf from the main branch in the early fruiting (35 days) (Trani and Raij, 1997) and P contents were determined by the method of phospho-molybdic complex in reducing environment, according to EMBRAPA (2006).
h) Content of phosphorus in the soil after harvest: phosphorus content in the soil after harvest: after harvesting the fruit and eliminated the shoots of each plot were taken at random two samples of soil in the row and phosphorus extracted by Mehlich-1 (HCl 0.05 mol $L^{-1}$ $H_2SO_4$ + 0.0125 mol $L^{-1}$) in a soil extractor ratio 1:10 (5 $cm^3$ of TFSA and 50 mL of extraction solution) and analyzed by flame photometry. Based on the adjusted equation for commercial productivity, an economic analysis of the experiment was carried out. Costs with fertilizers, increase in productivity, cost-effective factors and economic return were calculated. Increase in productivity was calculated by the difference between the productivity obtained by the dose used and the productivity of the treatment without phosphorus. The cost-effective relation resulted in the division between the increase in production and the cost of the phosphatic fertilizer. The economic return was obtained by multiplying the value of the cost-effective relation by the price of 1 t of commercial watermelon (R$ 180,00) or R$ 0,18 $kg^{-1}$, which corresponded to the average price of sales of the fruits intended for the domestic market. To calculate the costs with fertilizers, the cost of a unit of $P_2O_5$ (R$ $kg^{-1}$) was used, and a bag of 25 kg of triple superphosphate was acquired at the cost of R$ 50,95. The fixed cost, which corresponds to the sum of production and management costs, was not considered, because it is the same for all treatments. The data was submitted to a variance analysis (F test), using the statistics software SAS (SAS System, version 9.0). The regression equations were obtained by the table curve (Jandel Scientific, 1991), except for the total and commercial productivity, where the SAEG 9.0 (SAEG, 2005) software was used, due to the specificity of the equations.

## RESULTS AND DISCUSSION

### Content of phosphorus in the soil after harvest

The content of phosphorus (P) in the soil, assessed after the harvest of fruits was only influenced by the factor dose of phosphorus ($p < 0.01$) (Table 2), and as a higher dose was applied, the greater availability of P for watermelon was observed (Figure 1). Sandy textured soils have a better balance between content and P availability to plants, not being affected by the mounting process due to its lower content of clay, iron and aluminum oxides and calcium (Corrêa et al., 2011).

### Content of phosphorus in the leaf

Differently from the P content in the soil, greater doses of P did not influence the leaf content of phosphorus ($p >0.6$), which was affected only by the cultivar factor ($p< 0.01$) (Table 2). 'Top Gun' presented an average of 5.7 g $kg^{-1}$ of P, which was 26.7% higher than the leaf content observed in 'Olímpia' (4.5 g $kg^{-1}$). However, both contents are within the range from 3 to 7 g $kg^{-1}$ considered appropriate for the watermelon, according to Trani and Raij (1997). The increase in production provided by higher phosphorus availability in soil, resulted in a greater dilution of the phosphorus in the leaf tissue which contributed to the maintenance of its concentration (Larcher, 2004). The leaf contents of phosphorus range from 4.6 to 7.2 g $kg^{-1}$, for treatments with doses of 0 and 180 kg $ha^{-1}$ of $P_2O_5$, respectively. Even plants that did not receive the application of phosphorus had a leaf content within the range considered appropriate for the watermelon (Trani and Raij, 1997). Maluki et al. (2015), who evaluated doses of nitrogen and phosphorus in the cultivar Sugar baby during flowering and beginning of fructification, did not observe an increase in the leaf content of P in relation to the increase of phosphate fertilization either.

### Number of fruits per plant, total and commercial

Both number of fruits (NF) as to commercial fruit (NFC) per plant was not verified interaction between P doses and cultivars ($p> 0.49$ and $p> 0.95$ respectively). NF and NFC were influenced only by the dose of P (both with p

**Table 2.** Content of phosphorus in the soil (PS) and in the leaf (PF), fruits mass (MF), commercial fruits mass (MFC), number of fruits (NF), number of commercial fruits (NFC), total productivity (PT) and commercial productivity (PC) of the watermelon in relation to the doses of phosphorus and their respective averages for each cultivar.

| Doses of $P_2O_5$ (kg ha$^{-1}$) | PS (mg dm$^{-3}$) | PF (g kg$^{-1}$) | MF (kg) | MFC (kg) | NF (fruits plant$^{-1}$) | NFC (fruits plant$^{-1}$) | PT (kg ha$^{-1}$) | PC (kg ha$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| 0 | 9.8 | 4.46 | 8.01 | 8.32 | 1.22 | 1.13 | 48891.8 | 46967.3 |
| 45 | 30.0 | 5.09 | 8.78 | 9.10 | 1.72 | 1.60 | 74793.0 | 71968.9 |
| 90 | 42.3 | 5.52 | 7.90 | 8.76 | 2.07 | 1.66 | 79891.3 | 71586.0 |
| 135 | 63.0 | 5.03 | 8.34 | 8.98 | 1.91 | 1.66 | 78881.4 | 74402.6 |
| 180 | 95.9 | 5.20 | 8.14 | 8.65 | 2.02 | 1.79 | 81573.9 | 76964.6 |
| 225 | 116.3 | 5.35 | 8.20 | 8.89 | 1.99 | 1.69 | 80228.3 | 74624.7 |
| Cultivars | | | | | | | | |
| Top Gun | 61.50 | 5.67 | 7.92 | 8.42 | 1.82 | 1.58 | 71352.0 | 66464.5 |
| Olímpia | 56.35 | 4.54 | 8.54 | 9.15 | 1.82 | 1.59 | 76734.6 | 72373.6 |
| Values of F | | | | | | | | |
| $P_2O_5$ (P) | 13.42$^{**}$ | 0.71$^{ns}$ | 1.26$^{ns}$ | 1.34$^{ns}$ | 6.30$^{**}$ | 4.71$^{*}$ | 8.92$^{**}$ | 7.09$^{**}$ |
| Cultivars (C) | 0.65$^{ns}$ | 10.34$^{*}$ | 7.75$^{*}$ | 14.06$^{**}$ | 0.00$^{ns}$ | 0.01$^{ns}$ | 2.47$^{ns}$ | 2.97$^{ns}$ |
| P x C | | 1.56$^{ns}$ | 1.48$^{ns}$ | 0.47$^{ns}$ | 0.89$^{ns}$ | 0.22$^{ns}$ | 0.6$^{ns}$ | 0.46$^{ns}$ |
| SD | 22.14 | 1.22 | 0.78 | 0.67 | 0.35 | 0.30 | 11.872.81 | 11.870.38 |
| CV | 37.58 | 24.02 | 9.48 | 7.73 | 19.65 | 19.18 | 16.03 | 17.10 |

$^{ns}$ - non-significant; $^{*}$ - Significant ($p < 0.05$); $^{**}$ - Significant ($p < 0.01$).

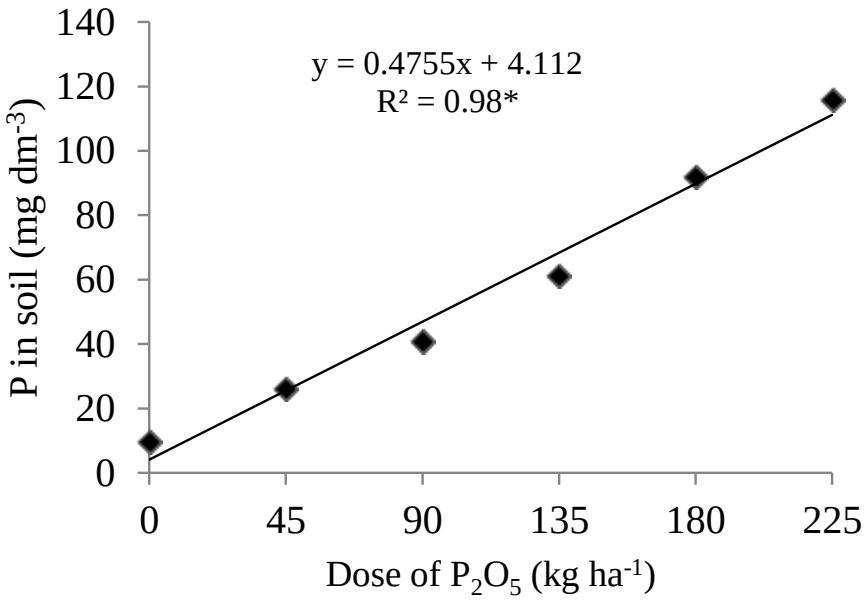


**Figure 1.** Content of phosphorus in the soil, assessed after the harvest of fruits, of both cultivars, in relation to the dose of phosphorus. Mossoró-RN, 2012.

<0.01) (Table 2), and there was adjustment in the linear plateau model of regression to the means of the characteristics in function of the dose of P. Maximums of NF (2 per plant) and NFC (1.7 per plant) were obtained with 77.22 and 54.75 kg ha$^{-1}$ of $P_2O_5$, respectively (Figure 2A and B). These results were 58.9 and 50.69% superior to the NF and NFC when there was no application of phosphorus. Leão et al. (2008), when evaluating the levels of chemical and organic fertilization in cultivar Crimson Sweet, found no interaction between these factors and obtained a maximum of 1.5 fruit per plant. This difference is because the current hybrids of watermelon are more productive than the cultivars of open pollination, being the NF one of the factors that contributes to that (Leão et al., 2008). The lack of response from NF and NFC above the doses of 77.22 and 54.75 kg ha$^{-1}$ of $P_2O_5$, respectively, can be an indication of maximum efficiency of the cultivar regarding

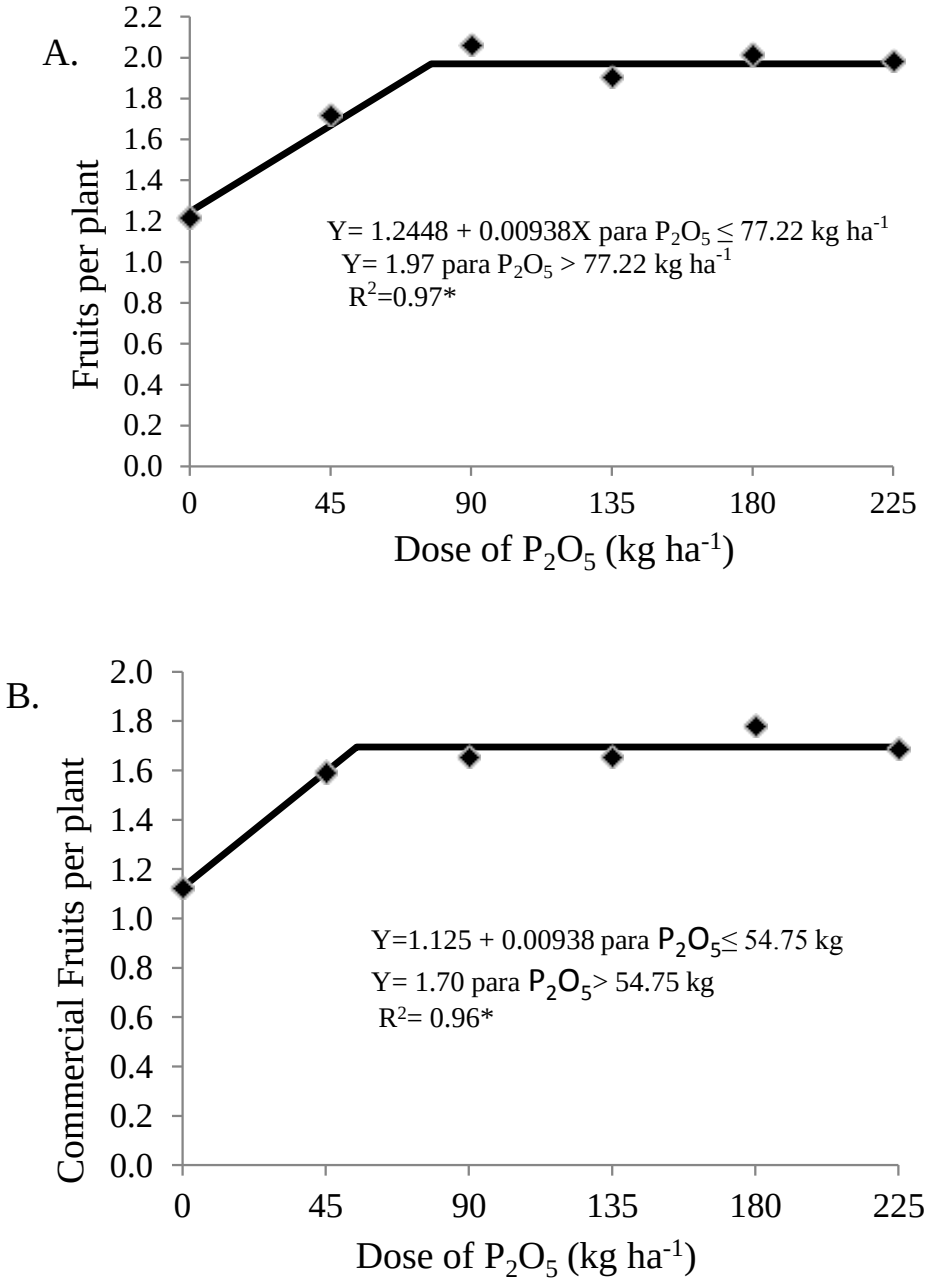


**Figure 2.** Number of fruits (A) and commercial fruits (B) per plant of both watermelon cultivars, in relation to the dose of phosphorus. Mossoró-RN, 2012.

absorption and/or use of this nutrient (Meng et al., 2014). Similar results were also found by Freitas Júnior et al. (2008).

**Total and commercial productivity**

The total (PT) and commercial (PC) productivity of fruits were influenced only by the dose of phosphorus (Table 2). There were adjustments in the linear plateau model of regression for these characteristics (Figure 3A and B). PT (80.14 t $ha^{-1}$) and PC (74.39 t $ha^{-1}$) maximums were obtained with 54.3 and 49.4 kg $ha^{-1}$, respectively. These doses provided increase of 63.9 and 63.1%, respectively, in the PT and PC treatment that did not receive phosphate fertilization. The dose that provided greater PC was close to the one that provided greater NFC per plant. These results are in accordance with the ones obtained by Hochmuth et al. (1993), who saw that the linear plateau model better represents the response to

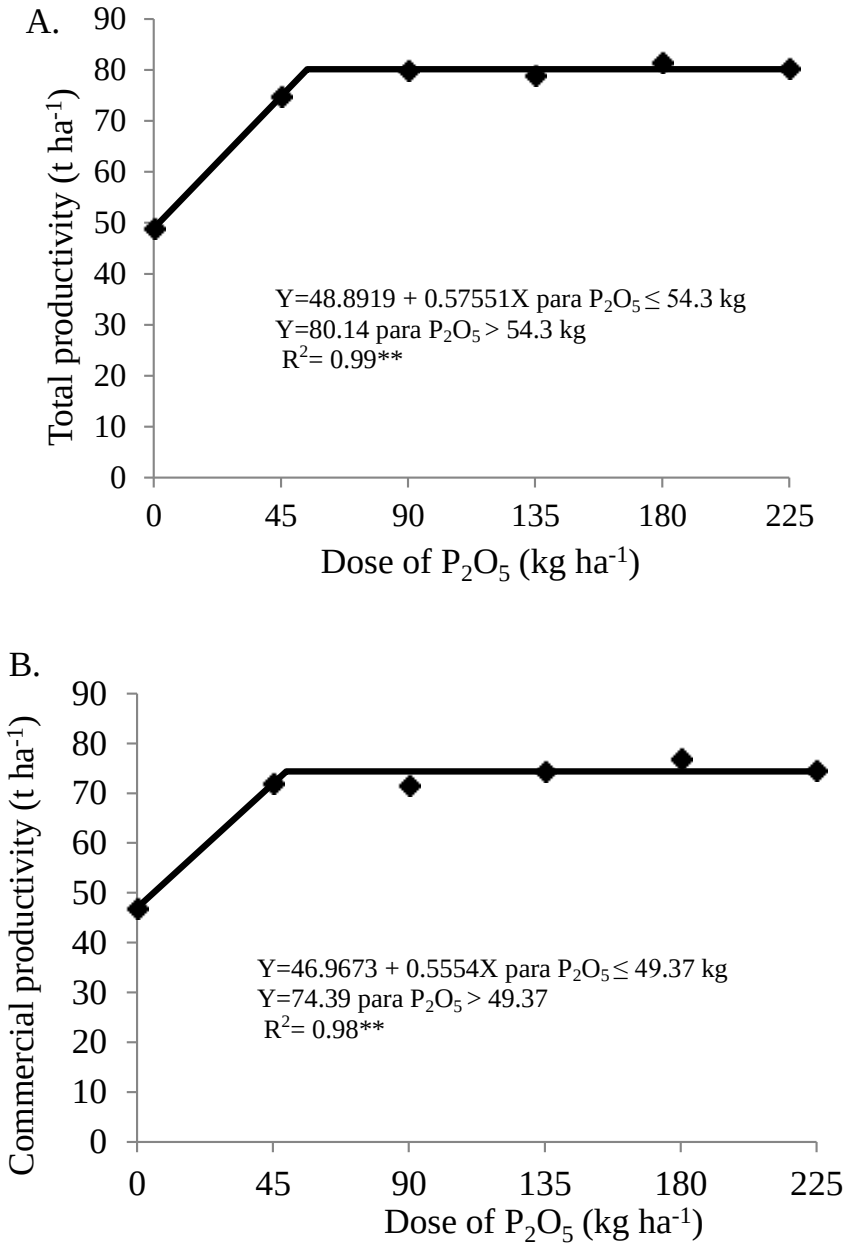


**Figure 3.** Total productivity (A) and commercial productivity (B) of both watermelon cultivars, in relation to the dose of phosphorus. Mossoró, 2012.

phosphate fertilization in soils with low content of phosphorus. These authors concluded that for two sites with low contents of phosphorus in the soil (between 5 and 6 mg $kg^{-1}$ - the solution extracted 0.0125 mol $L^{-1}$ $H_2SO_4$ and 0.050 mol $L^{-1}$ HCl), with only 60 kg $ha^{-1}$ of $P_2O_5$, 78.8% was obtained from the maximum production of 'Royal Jubilee' watermelon. However, the dose that maximized the PC differs from the one found by Leão et al. (2008), who obtained 22.5 t $ha^{-1}$ with 360 kg $ha^{-1}$ of $P_2O_5$, and also from Silva et al. (2014), whose maximum productivity was 23.84 t $ha^{-1}$ with the dose of 218 kg $ha^{-1}$ of $P_2O_5$.

These differences can be attributed both to the genetic variability and the type of mathematical model chosen to determine the production behavior, seeing that the quadratic models tend to overrate the dose of fertilizers required to reach maximum production (Cerrato and Blackmer, 1990). The high PT and PC obtained in this investigations can be attributed to the optimum conditions during cultivation, both in handling and climate, aligned with low incidence of pests and diseases. These productivities confirm the productive potential of

**Table 3.** Content of soluble solids (SS), peel thickness (EC), titratable acidity (ATT) and pulp firmness (FP) of the watermelon in relation to the doses of phosphorus and their respective averages for each cultivar.

| Doses of $P_2O_5$ | SS | EC | ATT | FP |
|---|---|---|---|---|
| (kg $ha^{-1}$) | (ºBrix) | (mm) | (%) | (N) |
| 0 | 10.7 | 14.00 | 0.136 | 9.05 |
| 45 | 10.4 | 13.00 | 0.127 | 9.19 |
| 90 | 10.3 | 13.81 | 0.134 | 10.04 |
| 135 | 10.2 | 14.13 | 0.138 | 9.66 |
| 180 | 10.7 | 13.25 | 0.143 | 9.28 |
| 225 | 10.2 | 13.88 | 0.140 | 10.34 |
| Cultivars | | | | |
| Top Gun | 10.7 | 13.25 | 0.137 | 9.76 |
| Olímpia | 10.2 | 14.10 | 0.136 | 9.43 |
| Values of F | | | | |
| $P_2O_5$ (P) | $1.01^{ns}$ | $1.00^{ns}$ | $1.81^{ns}$ | $1.32^{ns}$ |
| Cultivars (C) | $7.30^{**}$ | $5.47^{*}$ | $0.12^{ns}$ | $0.80^{ns}$ |
| P x C | $0.46^{ns}$ | $0.74^{ns}$ | $1.54^{ns}$ | $0.57^{ns}$ |
| SD | 0.67 | 1.26 | 0.01 | 1.27 |
| CV | 6.51 | 9.25 | 8.34 | 13.26 |

$^{ns}$ - non-significant; $^{*}$ - Significant ($p < 0.05$); $^{**}$ - Significant ($p < 0.01$).

watermelon hybrids and their efficiency in the use of phosphorus, showing that the producers can be applying excessive amounts of phosphorus, especially in sandy soils. The dose (54.3 kg $ha^{-1}$) that resulted in the maximum productivity was below the reference value adopted in this paper (90 kg $ha^{-1}$ of $P_2O_5$), which is the official recommendation of phosphorus for the state of Pernambuco, in the irrigated watermelon 'Crimson Sweet' crop, in soils with contents of phosphorus between 6 and 12 mg $dm^{-3}$ of P. With the dose that maximized the PC, by the end of the experiment, considering the adjustment obtained on Figure 1, 30 mg $dm^{-3}$ of P was still available for the plants, which can be considered an average content of P for the soil in which the experiment was done (CFSEMG, 1999).

## Mass of the fruits and commercial fruits

The mass of fruits and commercial fruits (MFC) were solely influenced by the cultivar factor (Table 2). The Olímpia cultivar had greater MF and MFC compared to the 'Top Gun' (Table 2). For both cultivars, the average of MFC was superior to 7 kg, considered a higher commercial value. These results corroborate the ones found by Freitas Júnior et al. (2008) and Silva et al. (2014), which did not see influence of doses of P for the mass of fruits.

## Attributes of fruit quality

For the attributes of fruit quality, there was significant effect only of cultivars in the content of soluble solids and peel thickness (Table 3). In general, the attributes of fruit quality present little environmental influence and high heritability (Ferreira et al., 2003). This can explain the lack of response to phosphate fertilization, regarding the attributes of quality of both hybrids. 'Top Gun' presented SS (10.7 °Brix) superior to 'Olímpia' (10.2 °Brix) (Table 3). The results overcome the value of 8.7 °Brix seen by Lima Neto et al. (2010), in the Crimson Sweet cultivar. Freitas Júnior et al. (2008), assessing the response of the 'Congo' watermelon to doses of phosphorus, did not verify the effect on the content of soluble solids either. The soluble solids (SS) represent the content of sugar in the fruits, so it is one of the characteristics that most contribute to the quality of the watermelon. The averages of SS seen for both cultivars were superior to 10 °Brix and, therefore, above the minimum required for the commercialization in the most demanding markets (9 °Brix) (Table 3). Currently, the farmers have preferred hybrids with greater content of SS because they can be a differential factor in the conquest of new markets and better prices, since part of the population chooses a better quality product, even if they have to pay a little more for that (Filgueiras et al., 2000). The peel thickness and the pulp firmness have strong influence in the resistance of fruits to handling and mechanic damage, and since the internal market is usually done in bulk, the fruits with greater peel thickness tend to have fewer problems during transportation (Barros et al., 2012). 'Olímpia''s peel thickness was superior (14.10 mm) to 'Top Gun' (13.25 mm). Values lower to these were seen by Barros et al. (2012), in the Crimson Sweet cultivar (12.57 mm). However, Lima Neto et al. (2010) found 17.3 mm for Crimson Sweet peel thickness, which shows that for the same cultivar there is great variation caused by

**Table 4.** Commercial productivity (PC), cost with phosphate fertilizer (CFF), productivity increase (IP), cost-effective (C/B) and economic return in relation to the dose of phosphorus in watermelon

| Doses of $P_2O_5$ (kg $ha^{-1}$) | PC (t) | CFF (R$) | IP (t) | C/B t/R$ | Return (b/a*180) (R$/R$) |
|---|---|---|---|---|---|
| 0 | 46.97 | 0.00 | - | - | - |
| 45 | 71.96 | 199.35 | 24.99 | 0.125 | 22.50 |
| 49.37[1] | 74.39 | 218.71 | 27.42 | 0.125 | 22.50 |
| 90 | 74.39 | 398.70 | 27.42 | 0.069 | 12.42 |
| 135 | 74.39 | 598.05 | 27.42 | 0.046 | 8.28 |
| 180 | 74.39 | 797.40 | 27.42 | 0.034 | 6.12 |
| 225 | 74.39 | 996.75 | 27.42 | 0.028 | 5.04 |

[1] Dose to maximize the productivity of commercial fruit.

the environment.

The average value of the titratable acidity of 0.137% (2.144 mmol $H^+$ 100 $mL^{-1}$) was lower to the ones obtained by Grangeiro and Cecílio Filho (2004), who found results in the interval of 0.247 to 0.256% of citric acid for the hybrid of watermelon Tide, as well as the ones seen by Lima Neto et al. (2010), which was 1.08% and Barros et al. (2012), who found 1.51%, both for Crimson Sweet. The pulp firmness, besides influencing the resistance to transportation and susceptibility to mechanic damage, also interferes in the post-harvest useful life. The average observed for pulp firmness was 9.59 N, a value lower to the one found by Barros et al. (2012), who found an average of 21.85 N for the Crimson Sweet watermelon.

## Economic analysis

The dose responsible for the maximum PC (49.37 kg $ha^{-1}$ $P_2O_5$) provided a return of R $ 22.50 for each real invested in fertilizer. Doses greater than 49.37 kg ha-1 $P_2O_5$ had significant decreases in economic returns, and the dose of 225 kg $ha^{-1}$ $P_2O_5$ reached R $ 5.04 (Table 4). The phosphorus fertilization has brought positive results for both soil fertility and for the watermelon productivity, but did not influence the quality.

## Conclusions

The response of cultivars of Olimpia and Top Gun watermelons to the increase in provision of P to the plants was similar, with maximum commercial productivity reached with 49.37 kg $ha^{-1}$ of $P_2O_5$. The increase in the dose of phosphorus from 0 to 225 kg $ha^{-1}$ of $P_2O_5$, in Red Yellow Argisol with 6.4 mg $dm^{-3}$ of P, does not influence the quality of watermelon fruits. The maximum economic return for the crop of watermelon in Red Yellow Argisol with 6.4 mg $dm^{-3}$ of P is reached with the dose of 49.37 kg $ha^{-1}$ of $P_2O_5$.

## Conflict of interests

The authors have not declared any conflict of interests.

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# Growth indexes, production and tolerance of peanut irrigated with saline water and bovine biofertilizer

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**Peanut (*Arachis hypogaea*) is one of the most cultivated oil plant worldwide since it is widely consumed as food. In this sense, this work aims to evaluate the growth, production and tolerance of peanuts under the effect of different levels of electrical conductivities in irrigation water and bovine biofertilizer. The experiment was conducted in a greenhouse located at the State University of Paraíba - Campus IV, municipality of Catolé do Rocha, Paraíba, Brazil. The experimental design was completely randomized with a factorial arrangement of 4 x 2, with six repetitions. There are two treatments: the first consisted of the combination of the electrical conductivity (ECw) of 0.5; 1.5; 3.0 and 4.5 dS $m^{-1}$ in irrigation water and the second is the application of bovine biofertilizer (with and without). Absolute growth rates and relative plant height, stem diameter and leaf area, number of pods per plant, 100 seed weight, number of seeds per plant, seed weight, seed mass + grains, root dry mass, shoot and total and tolerance index were assessed. From the results obtained, it can be concluded that the electrical conductivity of the irrigation water from 0.5 dS $m^{-1}$ significantly reduced the growth and production of *A. hypogaea*, however the application of bovine biofertilizer increased the results obtained.**

**Key words:** *Arachis hypogaea* L., salinity, organic input.

## INTRODUCTION

Peanut (*Arachis hypogaea* L.) is an important plant cultivated in large scale because of the great variability of products which it originates, especially in food and oil production (Duarte et al., 2013). In addition, the culture excels in crop production because its morphological and physiological characteristics help it to adapt well to dry

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soil. In the Northeastern region of Brazil, peanut is largely grown in family farming. However, there are great risks in its cultivation, due to the low rainfall in this region, which results in water deficit and low availability of good quality water (Graciano et al., 2011).

In order to raise the productivity of crops, several factors must be met, including the irrigation water quality; and among the characteristics that determine the irrigation water quality, soluble salts or salinity concentration is one of the main factors limiting the growth and development of some crops (Bezerra et al., 2010). In addition, irrigation management is a crucial factor in the preservation of natural resources, as it negatively affects the soil and can creat soil salinity.

One of the main challenges in agricultural production today is the development of cultural management techniques that allow the use of lower quality water in agriculture, once soil salinity or water negatively affects the development of plants at different stages (Guimarães et al., 2013). Thus, the use of organic inputs, such as bovine biofertilizer can be an alternative to reduce deleterious effects of salt stress as well as to make possible the cultivation of plants in saline environments.

Several studies have shown the harmful effects of saline irrigation water on the peanut crop (Correia et al., 2009; Graciano et al.; 2011; Santos et al., 2012; Sousa et al., 2012) and other oilseeds like castor bean (Nobre et al., 2013, 2014; Santos et al., 2013; Lima et al., 2014; Sá et al., 2016;), jatropha (Oliveira et al., 2013), sunflower (Morais et al., 2011; Maciel et al., 2012), and legumes like beans (Neves et al., 2008; Garcia et al., 2010; Bezerra et al., 2010; Aydin et al., 2012). However, most studies have not shown the effect of biofertilizers as a way to mitigate the effects of salt stress on peanuts, mainly in the reproductive phase.

The positive effects of organic inputs in the recovery of saline soils have been demonstrated (El-Dardiry, 2007; Miranda et al., 2011), including bovine biofertilizer. It has a positive action by improving the soil quality in terms of aeration, dynamic air and water in soil (Mavi et al., 2012), also by the possibility of complex substances derived from the organic matter to mitigate depressive effects of water salinity on plants (Aidyn et al., 2012). Furthermore, bovine biofertilizer has a positive action, presenting in its composition many beneficial substances including humic substances which promote the reduction of the osmotic potential of soil solution and thus stimulate the uptake of water and nutrients by plants in salty environments.

This study aims to evaluate the growth, production and tolerance of peanuts under the effect of electrical conductivities in irrigation water and bovine biofertilizer.

## MATERIALS AND METHODS

The experiment was conducted from September to December 2015 in the Humanities and Agricultural Sciences Center, Department of Agricultural and Exact State University of Paraíba (UEPB), Catolé do Rocha-PB, Brazil (6° 20'38 " S, 37° 44'48 "W); its altitude is 275 m. The climate of the city, according to Köppen classification, is BSW' type, that is, hot and dry steppe; its average monthly temperatures exceed 18°C throughout the year.

The experimental design was completely randomized with a factorial arrangement of 4 x 2, with six repetitions. There are two treatments: the first consisted of the combination of the electrical conductivity (ECw) of 0.5; 1.5; 3.0 and 4.5 dS $m^{-1}$ in irrigation water and the second is the application of bovine biofertilizer (with and without). The experimental units consisted of two plants grown in pots with a capacity of 15 $dm^3$.

The water used for irrigation showed an electrical conductivity of 1 dS $m^{-1}$. The water analysis was carried out by the Irrigation and Salinity Laboratory (LIS) of the Center for Technology and Natural Resources of the Federal University of Campina Grande – UFCG. It had the following chemical characteristics: pH ($H_2O$) = 7.53; $Ca^{2+}$ = 2.30 $cmol_c dm^{-3}$; $Mg^{2+}$ = 1.56 $cmol_c dm^{-3}$; Na = 4.00 $cmol_c dm^{-3}$; $K^+$ = 0.02 $cmol_c dm^{-3}$; chloride = 3.90 $cmol_c dm^{-3}$; carbonate = 0.57 $cmol_c dm^{-3}$; bicarbonate = 3.85 $cmol_c dm^{-3}$; RAS = 2.88 $(mmol_c l^{-1})^{1/2}$.

A soil classified as Fluvent sandy clay loam texture was used. Samples were collected from 0-20 cm layer in native area located on the campus of UEPB. The soil sample used for filling the polyethylene vessel was removed and a subsample was chemically analyzed. The following characteristics were obtained: $Ca^{2+}$ = 4.63 $cmol_c dm^{-3}$; $Mg^{2+}$ = 2.39 $cmol_c dm^{-3}$; $Na^+$ = 0.30 $cmol_c dm^{-3}$; $K^+$ = 0.76 $cmol_c dm^{-3}$; SB = 8.08 $cmol_c dm^{-3}$; $H^+$ = 0.00 $cmol_c dm^{-3}$; $Al^{3+}$ = 0.00 $cmol_c dm^{-3}$; cation exchange capacity = 8.08 and organic matter = 1.88 g $kg^{-1}$.

The biofertilizer was obtained by anaerobic fermentation, that is, in a hermetically sealed environment. To release methane at one end of a thin hose, the upper base of each digester was coupled and the other end was immersed in a container of water. For the preparation of biofertilizer we used 70 kg of manure from dairy cows and 120 L of water; 5 kg of sugar and 5 L of milk were added to speed up the metabolism of the bacteria (Silva et al., 2012).

The biofertilizer was applied 15 days after emergence at 10% of the volume of the vessels, and later in 15 - day interval, 6 applications were made. Prior to application, the biofertilizer was subjected to screening and filtering process to reduce the risk of clogging of the sieve watering holes. The biofertilizer was analyzed and had the following chemical characteristics (Table 1).

The different levels of electrical conductivity of water (ECw) were obtained by the addition of sodium chloride (NaCl) water from the local supply system according to Rhoades et al. (2000), and the quantity of salts (Q) was determined by the following equation: Q (mg $L^{-1}$) = ECw x 640, wherein ECw (dS $m^{-1}$) is the desired value of electric conductivity. Water chosen as control - $S_1$ (0.5 dS $m^{-1}$) stems from an Amazonas well of supply, located near the experimental area UEPB. Treatments with the different electrical conductivities of irrigation water began 15 days after emergence and until harvest.

Seeds were sown directly into pots with 3 in each pot. They emerged in 15 days, and were thinned to only the strongest plant. The growth of peanut was assessed at 30, 70 and 90 days after sowing (DAS) by measuring height of the plant, number of leaves, stem diameter and leaf area. From the monthly average values of plant height, stem diameter and leaf area, their respective absolute growth rate (AGR), relative growth rates (RGR) and shoot root ratio were calculated according to Benincasa (2003).

At 90 DAS during harvest, the number of pods per plant, 100 seed weight, number of seeds per plant, seed weight, seed mass + grains were also evaluated. In addition, there were also evaluated the dry mass of root, shoot and total dry mass. The dry matter of root, stem, shoot and total were determined after fresh weight was obtained for approximately 48h. They were circulated in air at 60°C

**Table 1.** Chemical attributes of liquid biofertilizers used in the experiment. Catolé do Rocha-PB, UEPB 2015.

| Chemical properties | Obtained values |
|---|---|
| pH | 4.68 |
| EC (dS $m^{-1}$) | 4.70 |
| Nitrogen (%) | 1.00 |
| Phosphorus (mg $dm^{-3}$) | 296.20 |
| Potassium ($cmol_c dm^{-3}$) | 0.71 |
| Calcium ($cmol_c dm^{-3}$) | 3.75 |
| Magnesium ($cmol_c dm^{-3}$) | 3.30 |
| Sodium ($cmol_c dm^{-3}$) | 1.14 |
| Sulfur ($cmol_c dm^{-3}$) | 14.45 |

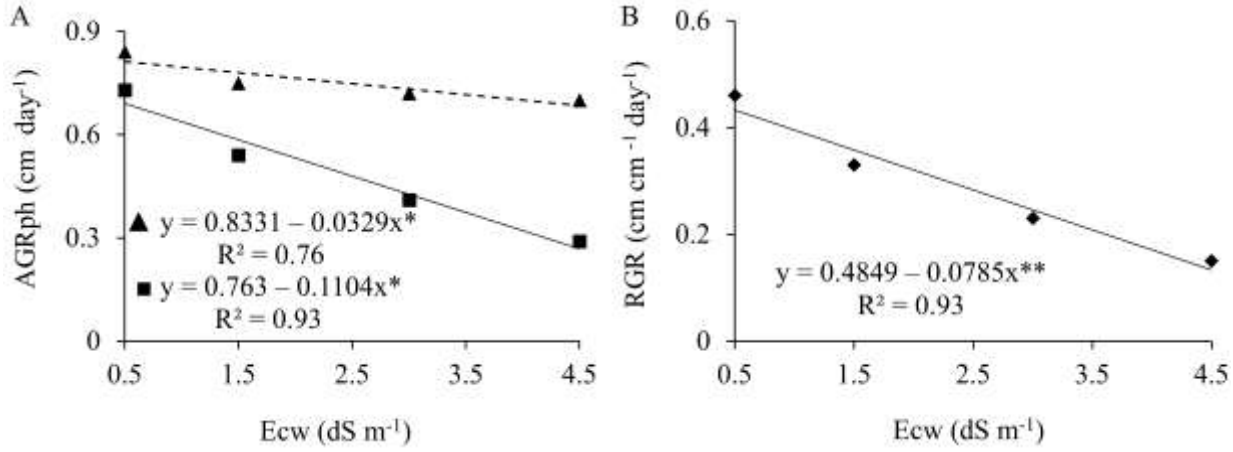


**Figure 1.** Absolute growth rate - AGRph (A) at 30 to 70 days after sowing (DAS) and relative - RGRph (B) high peanut under the effect of electrical conductivity irrigation water (▲) and without (■) bovine biofertilizer.

until a constant weight was obtained. Then they were weighed on a precision scale of 0.0001. The total dry matter production data were used to calculate the percentages partitioned between vegetative organs and the rate of salinity tolerance, comparing the data from saline treatments with the control (ECw = 0.5 dS $m^{-1}$) according to the methodology of Aquino et al. (2007).

Data were evaluated by analysis of variance of F test at 0.05 and 0.01 probability; and for significance, there was linear and quadratic polynomial regression analysis using the statistical software SISVAR 5.0 (Ferreira, 2011).

## RESULTS AND DISCUSSION

There was a significant effect of electrical conductivity in irrigation water (ECw) on all variables except for the relative growth rate of plant height (RGRph) at 70 to 90 DAS; absolute growth rate (AGRsd) and relative growth rate (RGRsd) at 70 to 90 DAS. The biofertilizer was only significantly effective on the absolute growth rate in the plant height (AGRph) at 70 to 90 DAS.There was noticed a significant effect of ECw x bovine biofertilizer interaction on AGRph and RGRsd variables at 30-70 DAS and on AGRph at 70 to 90 DAS.

The ECw negatively influenced the absolute growth rate of the plant height (AGRph) at 30to 70 DAS, showing a decrease of 0.0329 cm $day^{-1}$ when the plants received bovine biofertilizers; when without bovine biofertilizer was not applied, there was a reduction of 0.1104 cm $day^{-1}$ (Figure 1A). It was also observed that most AGRph was obtained in plants irrigated with water of 0.5 dS $m^{-1}$ at 0.84 cm $day^{-1}$ and bovine biofertilizer; there was lower AGRph when the plants were irrigated with water of 4.5 dS $m^{-1}$ and when bovine biofertilizer was not applied. This occurred at a rate of 0.29 cm $day^{-1}$.

For the relative growth rate of plant height (RGRph), linear decrease of 0.0785 cm $cm^{-1}$ $day^{-1}$ per unit

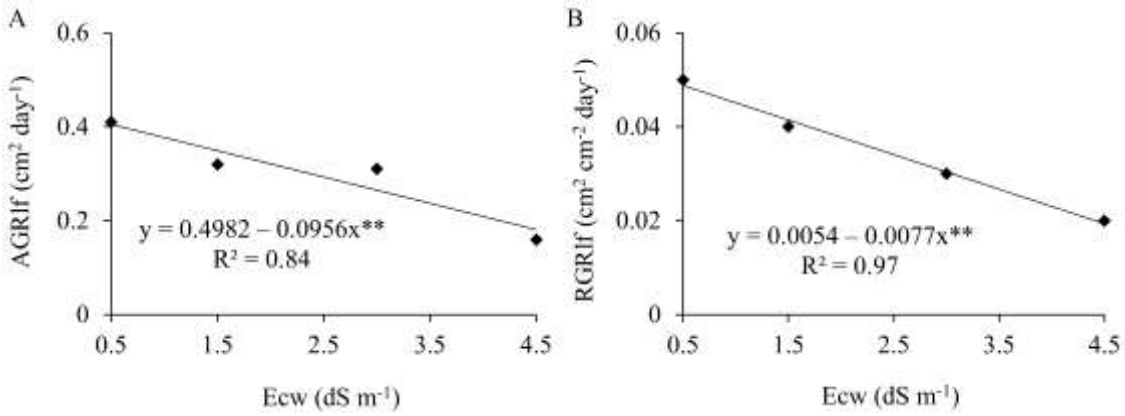


**Figure 2.** Absolute growth rate - AGRlf (A) and relative - RGRlf (B) of peanuts leaf area under the effect of electrical conductivity of irrigation water in the period 30-70 days after sowing (DAS).

increased ECw at 30-60 DAS. The largest RGRph was obtained when the plants were irrigated with water of 0.5 dS $m^{-1}$; its value was 0.46 $cm^{-1}$ $day^{-1}$ cm, while the lowest was 0.15 cm RGRph $cm^{-1}$ $day^{-1}$ when the plants were irrigated with water of 4.5 dS $m^{-1}$ (Figure 1B).

Several studies have shown that salinity reduces absolute growth rate and relative plant height. This was observed in the culture of castor bean by Lima et al. (2014), who found that AGRph of castor bean decreased with increasing ECw, reaching a 2.47 cm $day^{-1}$ with water of 0.3 dS $m^{-1}$. Nobre et al. (2014) found that RGRph had a linear increase in the order of 1.63% per unit increase in the ECw, that is, increment of 6.52% on RGRph plants irrigated with water of 4.4 $dSm^{-1}$ as compared to the control (0.4 dS $m^{-1)}$.

One of the causes of reduction in growth rate is decreased turgor of the plant, possibly by reducing the size of plants and leaves; thus plants decrease radiation gathering area, absorb less nutrients from the soil and make less $CO_2$ exchange with the environment. This reduces their photosynthetic potential and consequently their productivity (Ávila et al., 2007).

It was observed that the ECw exerted significant effects on absolute growth rate (AGRlf) and relative (RGRlf) of leaf area of peanut plants at 30-70 DAS, where there was reduction of 0.0956 $cm^2$ $day^{-1}$ (Figure 2A) for AGRlf $cm^2$ and 0.0077 $cm^{-2}$ $day^{-1}$ (Figure 2B) with increasing ECw, respectively. So, the highest values (0.41 $cm^2$ $day^{-1}$ to AGRlf and 0.05 $cm^2$ $cm^{-2}$ $day^{-1}$ to RGRlf) were obtained when the plants were irrigated with water of low salinity (0.5 dS $m^{-1}$) than those irrigated with water of 4.5 dS $m^{-1}$.

Santos et al. (2013) found that AGRlf of castor bean was decreased with increasing ECw. They obtained the highest AGRlf (194 $cm^2$ $day^{-1}$) under irrigation water of 0.12 dS $m^{-1}$ and -51.4 $cm^2$ $day^{-1}$ and irrigation water of high salinity (4.8 dS $m^{-1}$). The same authors reported that the AGRlf was not affected by the saline of irrigation water; however, it affects negatively the regression model as a function of cultivation time for 80 DAE growth rate - 0.01 $cm^2$ $cm^{-2}$ $day^{-1}$.

The reduction in growth rate is caused by decreasing turgor, where as, small decreases in the water content and turgor can reduce growth rate or even prevent it completely (Echer et al., 2010). Furthermore, the decrease in relative growth rate of leaf area is also caused by the deleterious effect of the excess salts in the plant metabolism (Santos et al., 2013).

As the regression equation shows in Figure 3A, the model to which the best data were set was linear. In it, one can see a reduction in the absolute growth rate of stem diameter (AGRsd) (0.0072 mm $day^{-1}$) based on a unit increase of ECw; the maximum value obtained was 0.061 mm $day^{-1}$ when plants were irrigated with 0.5 $dSm^{-1}$ water. As for the relative growth rate of stem diameter (RGRsd), it can be observed a significant effect of the interaction between ECw x bovine biofertilizer. The reductions were 0.0008 mm $mm^{-1}$ $day^{-1}$ in plants that received biofertilizer and 0.0016 mm $mm^{-1}$ $day^{-1}$ without biofertilizer (Figure 3B).

Santos et al. (2013) found that AGRsd was reduced with increasing irrigation water salinity, which obtained the highest rate at 80 DAE with 4.8 dS $m^{-1}$ getting 2.95 and 0.006 mm $day^{-1}$ when the plants were irrigated with water of low salinity (0.12 dS $m^{-1}$). For RGRsd, the same authors observed a decrease during the evaluation period, regardless of the salt content of irrigation water, which obtained the highest relative growth rate in stem diameter of 0.03 mm $mm^{-1}$ $day^{-1}$ in irrigated plants with low salinity water (0.12 dS $m^{-1}$) and water of higher salinity; the growth rate of stem diameter was 0.02 mm $mm^{-1}$ $day^{-1}$.

It was observed that ECw had negative influence on absolute growth rate of plant height (AGRph) at 70-90 DAS; increased water salinity decreased the AGRph

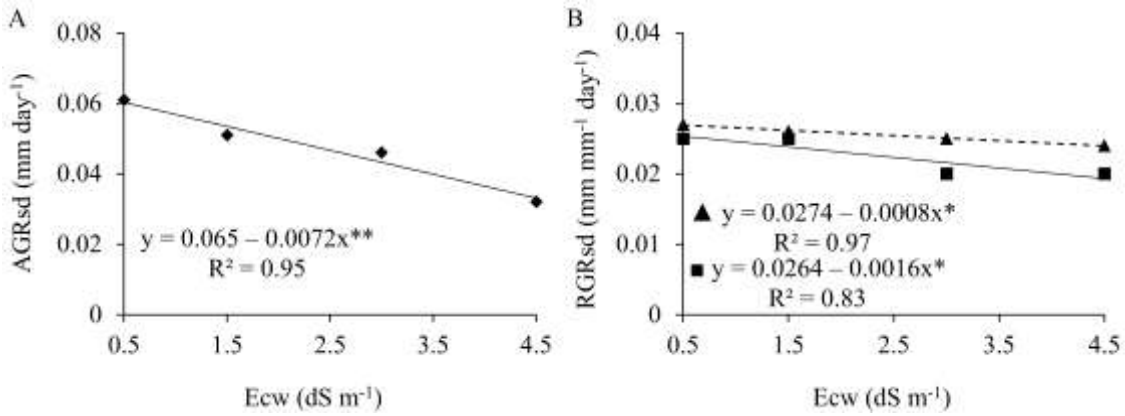


**Figure 3.** Absolute growth rate - AGRlf (A) and relative - RGRlf (B) diameter of peanut under the effect of electrical conductivity of stem of irrigation water with (▲) and without (■) bovine biofertilizer in the period from 30 to 70 days after sowing (DAS).

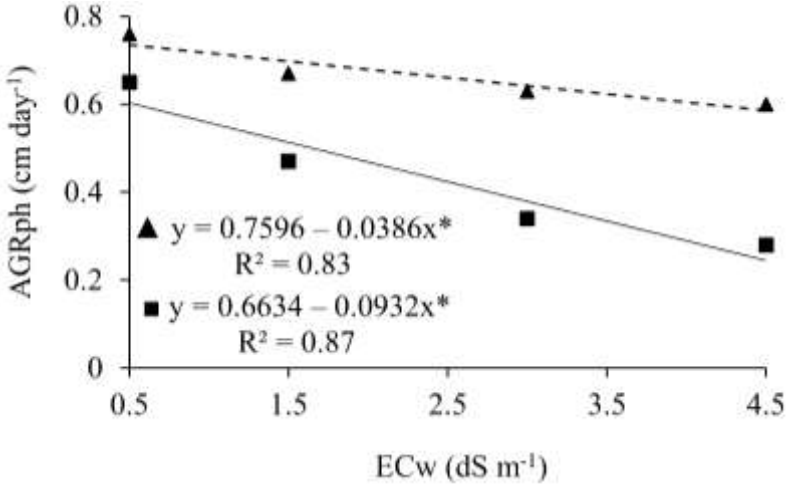


**Figure 4.** Absolute growth rate of plant height - AGRph peanuts in the electrical conductivity effect of plant irrigation water with (▲) and without (■) bovine biofertilizer in the period 70-90 days after sowing (DAS).

0.0386 and 0.0932 cm day$^{-1}$ per unit increase of ECw on the treated plants with and without bovine biofertilizer, respectively. However, in irrigation water of low salinity (0.5 dS m$^{-1}$), plants that received bovine biofertilizer were superior in the results obtained (0.76 cm day$^{-1}$); while in irrigation water with high salinity (4.5 dS m$^{-1}$) and without bovine biofertilizer, lower values (0.28 cm day$^{-1}$) were noticed (Figure 4).

One of the deleterious effects of salinity is the reduction of plant growth. Therefore, one of the parameters used to evaluate the effects of salinity is the growth rate of the plant's ability to tolerate this stress (Correia et al., 2009; Garcia et al., 2010). Furthermore, salinity inhibits the growth of the plant height, stem diameter, leaf area, causing negative effects on absolute growth rate of each of the respective variables (Santos et al., 2013).

ECw exerted significant effects on absolute growth rate (AGRlf) and relative (RGRlf) of peanut leaf area at 70-90 DAS; there was a reduction with increasing ECw in the order of 0.0075 cm² day$^{-1}$ (Figure 5A) for AGRlf and 0.0074 cm² cm² day$^{-1}$ (Figure 5B) for AGRlf. When the peanut plant was subjected to ECw of 4.5 dS m$^{-1,}$ its AGRlf and RGRlf were lower compared to plants irrigated with low saline water (0.5 dS m$^{-1}$).

One of the parameters used to evaluate the effects of salt stress and plant capacity to overcome salinity is the growth rate and biomass production, since plant growth processes are particularly sensitive to salts (Morais et al.,

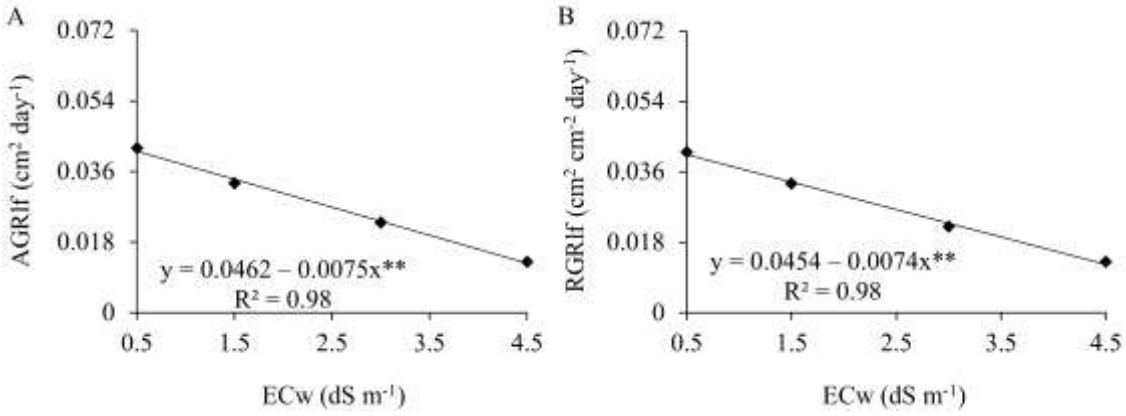


**Figure 5.** Absolute growth rate - AGRIf (A) and relative - RGRIf (B) of leaf area peanuts under the effect of electrical conductivity of irrigation water in the period 70-90 days after sowing (DAS).

2011). Reductions in relative growth rate of leaf area are mainly due to the effect of excess salts on plant metabolism (Santos et al., 2013).

ECw has significant effect on the variables; bovine biofertilizer had a significant effect on seed weight and tolerance index. There was also significant effect of ECw x B interaction on the variables, number of pods, weight of 100 seeds (W100S), number of seeds, seed mass and index of tolerance (IT).

It is shown in Figure 6A that the number of pods was reduced by increasing ECw; plants irrigated with water of 4.5 dS $m^{-1}$ without bovine biofertilizer (6.5 pods) had low values; while maximum value (25.5 pods) was obtained when the plants were irrigated with low salinity water (0.5 dS $m^{-1}$) under bovine biofertilizer. For number of seeds (Figure 6B), the irrigation water of 4.5 dS $m^{-1}$ linearly decreased this parameter; the lowest values of 20 seeds were obtained from the plants treated without bovine biofertilizer, and the largest number of seeds (54.5) was obtained in plants irrigated with water of 0.5 dS $m^{-1}$ and treated with bovine biofertilizer (Figure 6B).

Correia et al. (2009) found that salinity significantly affects the number of peanut fruits, with a total reduction of 36% in higher salinity irrigation water (ECw = 6.0 dS $m^{-1}$). Salt stress reduces plant growth, causing a decrease in osmotic potential and/or excessive accumulation of ions in the plasma and may induce ionic toxicity, nutritional imbalance or both. Thus, in order for the plants to adapt, the size of the leaves, transpiration surface and the exposed area to capture radiation were reduced. Thus, lower plants have less transpiration capacity, less potential to absorp nutrients from the soil solution and lower $CO_2$ exchange with the environment. This reduces their photosynthetic potential, and as a result, a lower plant productivity is noticed (Garcia et al., 2010).

Due to the presence of humic substances in bovine biofertilizer, as humic acids, a higher grain yield was registered when plants were treated with organic feedstock, since these substances increase cell division; and the permeability of cell membranes thus provides greater absorption of water and nutrients for plants exposed to salt stress and increased production of fruits (Khaled and Fawy, 2011).

Plants grown under saline water are likely to originate from seeds with low physiological quality. However, studies show that it does not happen often. It is essential for these data, since producers living in regions with a shortage of good quality water can use saline water for irrigation (Dantas et al., 2015). Moreover, the application of organic inputs, such as bovine biofertilizer can increase water retention capacity, soil aggregation and reduce bulk density (Mgbeze and Abu, 2010).

ECw negatively influenced the weight of 100 seeds (W100S) and seed mass of peanut plant. The regression equations show the data are linear, indicating a decrease of 4.05 g 8 48 g in 100 seed weight (Figure 7A) on the treated plants with and without biofertilizer, respectively and 2.96 and 4.68 g in seed weight (Figure 7B) in plants treated with and without biofertilizer, respectively. The biggest gains in W100S and seeds mass occurred in plants irrigated with low salinity water (0.5 dS $m^{-1}$), with 74.3 (W100S) and 63.75 g seeds mass.

Correia et al. (2009) found that the weight of 10 seeds had a total decrease of 78.3% in ECw 6.0 dS $m^{-1}$ in peanuts. The same authors associated these results to the deleterious effects of irrigation water salinity on the physiology of the plant. It promoted metabolic disorders, especially in relation to the absorption of water and nutrients from the soil, reduced leaf area, resulting in less photosynthetic surface area and lower crop yield. In the case of bean seeds, Neves et al. (2008) observed that irrigation with saline water (ECw 5.0 dS $m^{-1}$) started after germination and until the end of the bean cycle it did not influence the weight of 100 seeds.

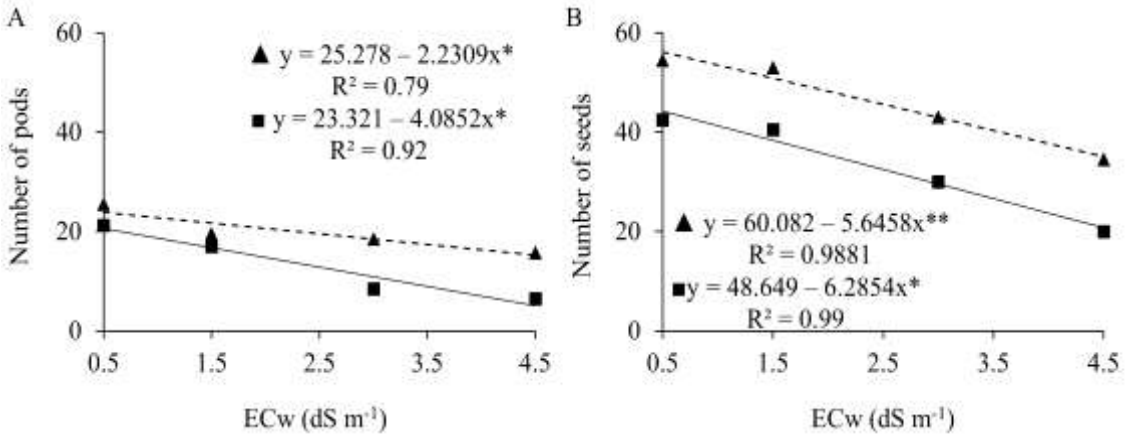


**Figure 6.** Number of pods (A) and seeds (B) under the effect of peanut electrical conductivity of the irrigation water with (▲) or without (■) bovine biofertilizer.

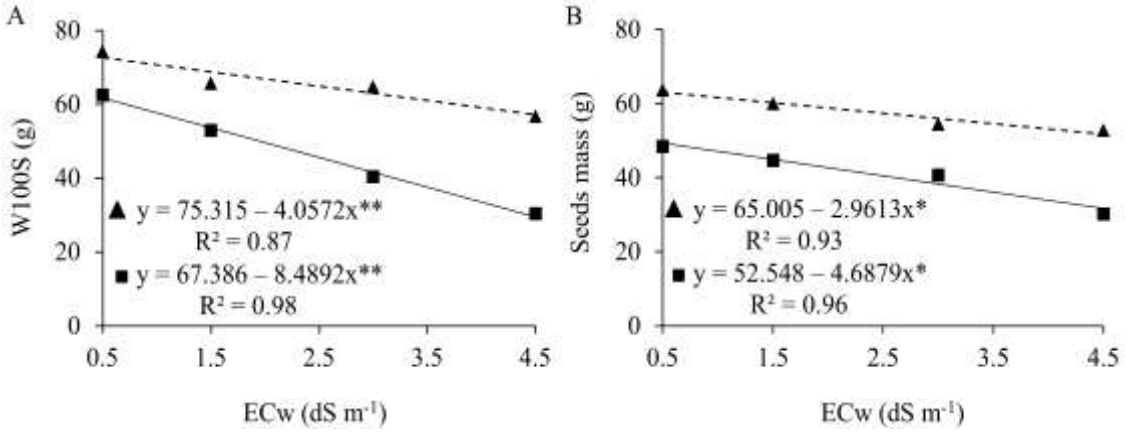


**Figure 7.** Weight of 100 seeds (A) and seed mass (B) of peanut under the effect of electrical conductivity of irrigation water with (▲) and without (■) bovine biofertilizer.

This increase in weight of 100 seeds and seed mass can be attributed to the beneficial action of bovine biofertilizer, as the organic feedstock operates in the physical improvement of the soil for root growth systems, as discussed by Mgbeze and Abu (2010) and Benbouali et al. (2013) and also to improve soil biological activity (Cha-Um and Kirdmanee, 2011).

It is observed that the mass of pods + grain (Figure 8A) showed a significant effect when subjected to different ECws and behaved linearly in decreasing order. It had minimum values in higher salinity levels (value 3.73 g); each unit increase in ECw levels decreased in the order of 0.55 g.

Regarding the plant dry matter (Figure 8B), a significant effect of ECw on root dry mass (RDM), shoot dry mass (SDW) and total dry mass (TDM) was registered, so that by increasing the ECw levels observed parameters decreased in the order of 1.56 g (RDM), 6.14 g (SDW) and 7.70 g (TDM). When the plants were irrigated with low salinity water (0.5 dS $m^{-1}$) there were shown the highest values of 14.23 g (RDM), 35.66 g (SDW) and 49.9 g (TDM) while under irrigation with high salinity (4.5 dS $m^{-1}$) lower values of 8.6g (RDM), 20.76 g (SDW) and 29.36 g (TDM) were observed.

The root dry mass, shoot dry weight, total dry mass and dry mass of pods + peanut kernels were reduced with increasing ECw (Santos et al., 2012). Sousa et al. (2012) studied the salt stress in peanut crop and concluded that the high concentration of salts in irrigation water reduced the root dry matter at 45 days after sowing. Graciano et al. (2011) found that root dry mass showed a significant increase of 84, 60 and 58% in plants subjected to treatments of 3.5, 6.0 and 8.5 dS $m^{-1}$ EC, respectively, compared to control (ECw 1.0 dS $m^{-1}$); however, for the

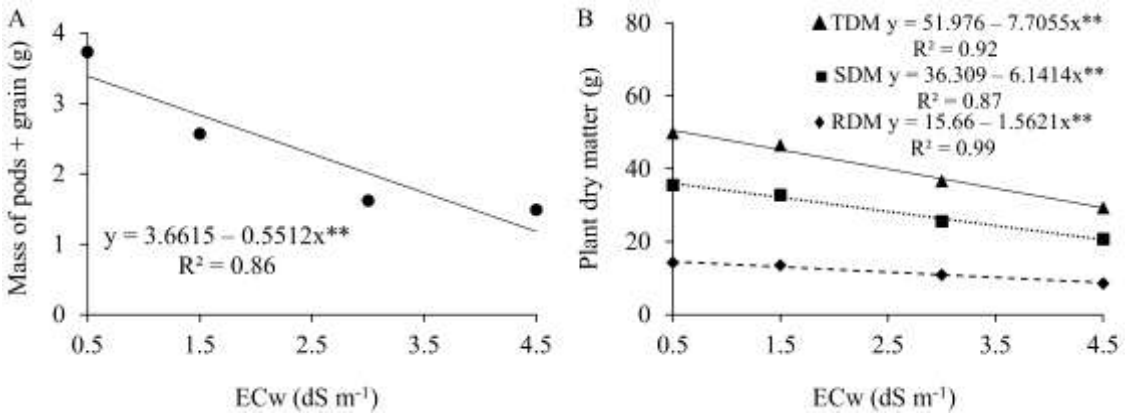


**Figure 8.** Pods + grain mass (A) and plant dry matter (B) of peanut under the effect of electrical conductivity of irrigation water, root dry mass (♦), shoot (■) and total (▲).

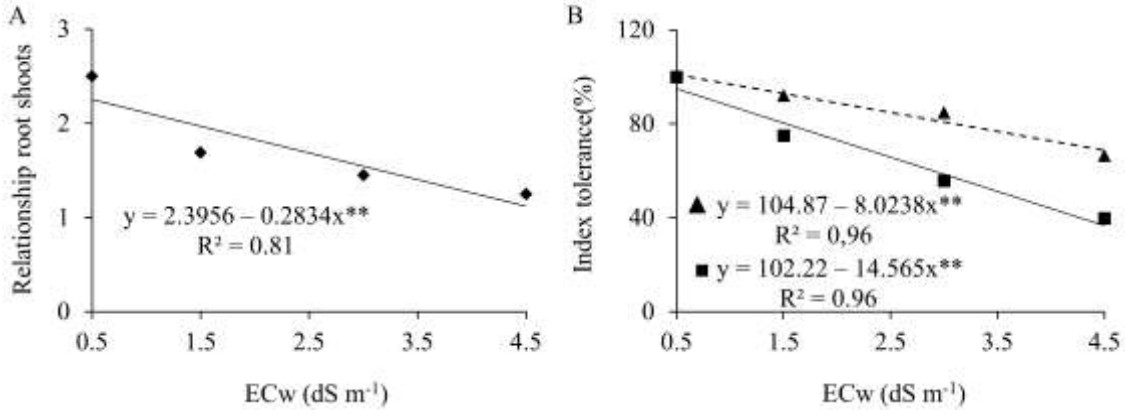


**Figure 9.** Relationship roots and shoots (A) and tolerance index (B) of peanut under the effect of electrical conductivity of irrigation water with (▲) and without (■) bovine biofertilizer.

total dry mass, the authors found no significant effects.

The lower production of dry mass of plants is due to the effects of salinity, reducing the availability of water to plants due to the decrease in the total water potential in the soil; and as a result, there is greater energy expenditure in plants for absorption of water (Leonardo et al., 2007). The effect of salinity on dry matter accumulation has been observed by several authors and different species of oil of agronomic interest such as sunflower (Morais et al., 2011; Maciel et al., 2012) peanut (Correia et al., 2009; Santos et al., 2012), jatropha (Oliveira et al., 2015) and castor bean (Nobre et al., 2013; Lima et al., 2014).

The same behavior of the above variables can be observed for the root shoot ratio, where the data set to decreasing linear model, down 0.28 each unit increase in the levels of ECw; the lowest values in the higher salinity identifying themselves (4.5 dS $m^{-1}$) with the value of 1.25 cm and the maximum value (2.5) when the plants were irrigated with water of low salinity (0.5 dS $m^{-1}$) (Figure 9A).

As it can be observed in Figure 9B, the peanut tolerance index decreased linearly, as the increment of ECw in which the obtained decreases from 8.02% to 14.56% per unit increase of ECw on the treated plants with and without biofertilizer bovine, respectively. Lower level of tolerance (39.75%) was observed when plants were irrigated with high salinity (4.5 dS $m^{-1}$) and without application of bovine biofertilizer while the highest rate of tolerance (100%) was observed in plants irrigated with low salinity (0.5 dS $m^{-1}$) and treated with bovine biofertilizer.

The relationship between root and shoots of groundnuts increased significantly by 75, 58 and 75% in ECw 3.5, 6.0 and 8.5 dS $m^{-1}$ in the control, respectively (Graciano et al., 2011). Sá et al. (2016) found that

increase in salinity levels caused linear reductions in salt tolerance index of all the varieties of castor bean, with 63.82% reductions to cultivate LA Guarani, 79.80, 75.51 and 77.91% for BRS Energy cultivars BRS Gabriela and IAC 028, respectively. Graciano et al. (2011) observed that peanut cv. BR1 is sensitive to salinity. However, they found that it tolerates salinity culture conditions, due to adaptation and resistance to salinity strategies such as development of root system and a small percentage of reducing shoot growth variables.

## Conclusions

The electrical conductivity in the irrigation water above 0.5 dS $m^{-1}$ negatively affects growth rate, production and tolerance of peanut. Bovine biofertilizer does not mitigate the effect of salt stress on peanuts; however, better results in growth rate, production and tolerance can be observed with the application of this input.

The interaction of electrical conductivity in the irrigation water and applying bovine biofertilizer has resulted in plant growth and a higher peanut production.

## Conflict of Interests

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Growth aspects and production of cotton under salt stress as a function of organic fertilizer

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**The cotton plant is a species cultivated in many parts of the world, with enormous economic importance. The objective of this study was to evaluate the effect of salinity levels in earthworm humus quantities function on the growth and production of cotton. The experiment was conducted in a greenhouse at the Campus IV in the State University of Paraiba, municipality of Catolé do Rocha-PB, Brazil. A completely randomized design with four replications, in a factorial 4 x 4 was used. The first factor consisted of salinity levels in irrigation water (0.8, 3, 4.5 and 6 dS $m^{-1}$) and the second factor of earthworm humus amounted to: 0, 1, 2 and 3 kg/plant. At the end of the experiment, the growth characteristics: plant height, stem diameter, leaf number, unit leaf area and leaf area of the plant were evaluated. The production: number of flower buds, number of bolls per plant, seed weight per boll, number of seeds per boll, 100 seed weight, total production, fiber production and seed production were also estimated. The interaction between salinity levels and quantities of earthworm humus did not affect the growth and production of cotton. The increase in salinity levels adversely affected the cotton crop. Largest earthworm humus quantities gave an increase in cotton production.**

**Key words:** *Gossipium hirsutum* r. *latifolium* H., Electrical conductivity of water, humus earthworm.

## INTRODUCTION

The cotton crop (*Gossipium hirsutum* L. r. *Latifolium* H.) stands out as one of the most important Brazilian agribusiness activities, and the herbaceous cotton is cultivated in more than fifteen countries (Oliveira et al., 2012). Although, a tolerant crop, the cotton can suffer substantial reductions in their growth, yield and quality when exposed to salt stress condition (Oliveira et al., 2008).

For most crops grown in semi-arid northeast, irrigation is important due to irregularities in rainfall and irrigation management which is crucial to achieving high production and quality of the products; however, the amount of water, the quality of the water used, particularly in relation to the concentration of soluble salts are important in irrigation (Oliveira et al., 2011).

Salinity is one of the abiotic stresses which affects the growth and productivity of plants (Nascimento et al., 2011; Blanco, 2008), affecting the osmotic potential of the soil solution, causing water stress and toxic effects on plants, which result in metabolism and nutritional disorders (Garcia et al., 2007; Sousa et al., 2010).

The work carried out with cotton in saline soil by Oliveira et al. (2012a) observed a reduction in which all characteristics with increasing irrigation water salinity had great reductions in leaf area (average 65.8%) and dry weight of vegetative parts (64%). Santos et al. (2016) also found that salinity irrigation water affects the growth and production of cotton.

In order to mitigate the effects of salt stress, organic inputs were applied. However, in the literature, there is a lack of studies related to earthworm humus effects and its use in crop plants under saline environment. In addition, due to the rising cost of mineral fertilizers and the growing environmental pollution, the use of organic waste in agriculture is an attractive option from an economic point of view, because of the carbon cycling and nutrients (Silva et al., 2010).

Organic fertilizers such as manure and earthworm humus are the most used among the small and medium producers of vegetables; however, the supply to the soil should consider the type, texture, structure and content of organic matter (Santos et al., 2006).

In this sense, the objective is to evaluate the effect of salinity levels in earthworm humus quantities function on growth and production of cotton.

## MATERIALS AND METHODS

The experiment was conducted in a greenhouse at the Center for Humanities and Agrarian State University of Paraíba (UEPB) in municipality of Catolé do Rocha-PB, Brazil (6° 20'38 "S, 37° 44'48" W) 275 m altitude. The climate of the city, according to Koppen classification, is the BSW type, that is, hot and dry steppe type, with average monthly temperatures exceeding 18°C throughout the year. A completely randomized design was adopted, with a factorial arrangement of 4 x 4, with four repetitions, corresponding to four levels of salinity of irrigation water (0.8, 3, 4.5 and 6 dS $m^{-1}$) according to the quantity of earthworm humus: (0, 1, 2 and 3 kg/plant).

**Table 1.** Physical and chemical attributes of earthworm humus used in the experiment. Catolé do Rocha-PB, UEPB 2014.

| Chemical properties | Values |
|---|---|
| pH $H_2O$ (1: 2.5) | 7.38 |
| Electrical conductivity (dS $m^{-1}$) | 2.11 |
| Calcium ($cmol_c$ $dm^{-3}$) | 3.54 |
| Magnesium ($cmol_c$ $dm^{-3}$) | 1.93 |
| Sodium ($cmol_c$ $dm^{-3}$) | 0.18 |
| Potassium ($cmol_c$ $dm^{-3}$) | 0.14 |
| S ($cmol_c$ $dm^{-3}$) | 5.79 |
| Hydrogen ($cmol_c$ $dm^{-3}$) | 0.00 |
| Aluminum ($cmol_c$ $dm^{-3}$) | 0.00 |
| Phosphorus ($cmol_c$ $dm^{-3}$) | 5.51 |

The water used for irrigation had electrical conductivity of 0.8 dS $m^{-1}$. Water analysis was performed and showed the following chemical characteristics: pH = 7.53; Ca = 2.30 $cmol_c$ $dm^{3}$; Mg = 1.56 $cmol_c$ $dm^{-3}$; Na = 4.00 $cmol_c$ $dm^{-3}$; K = 0.02 $cmol_c$ $dm^{-3}$; Chloride = 3.90 $cmol_c$ $dm^{-3}$; Carbonate = 0.57 $cmol_c$ $dm^{-3}$; Bicarbonate = 3.85 $cmol_c$ $dm^{-3}$; RAS (Soil Adsorption Ratio) = 2.88 $(mmol_c\ l^{-1})^{1/2}$.

The plants were cultivated in polyethylene pots with a capacity of 8.5 dm. It was used to fill the soil polyethylene pots and earthworm humus in the ratio of 2:1, and the soil was classified as Fluvisol of clayey sandy loam texture. The soil was analyzed and it showed the following characteristics: Ca = 4.63 $cmol_c$ $dm^{-3}$; Mg = 2.39 $cmol_c$ $dm^{-3}$; Na = 0.30 $cmol_c$ $dm^{-3}$; K = 0.76 $cmol_c$ $dm^{-3}$; Sum of bases - SB = 8.08 $cmol_c$ $dm^{-3}$; H = 0.00 $cmol_c$ $dm^{-3}$; Al = 0.00 $cmol_c$ $dm^{-3}$; cation exchange capacity (CTC) = 8.08 and = 1.88% organic matter. The earthworm humus used in filling had the characteristics shown in Table 1.

The different levels of salinity (ECw) were obtained by the addition of sodium chloride (NaCl) water from the local supply system according to Rhoades et al. (2000) and the quantity of the salt (Q) was determined by the equation:

$$Q\ (mg/L^{-1)} \times 640 = ECw \quad (1)$$

In that ECw (dS $m^{-1}$) is the desired value of the electrical conductivity of water. Water chosen as control - $S_1$ (0.8 dS $m^{-1}$) stems from a well located in the Amazons UEPB. The experimental units were composed of two plants, grown in plastic pots with a capacity of 8.5 $dm^3$. The seeds were sown in plastic pots with a capacity of 8.5 kg. The soil was sieved and mixed with earthworm compost in the ratio 2:1. At 14 days after sowing, there was thinning of plants keeping only the most vigorous.

At the end of the experiment, the growth characteristics and production plant height, stem diameter, number of leaves, unit leaf area, leaf area of the plant, number of flower buds, number of bolls per plant, seed weight per boll, number of seeds per boll, 100 seed

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weight, total production, fiber production and seed production were evaluated. The plant height was determined by a graduated tape measure in centimeters positioned at the stem base near the soil to the youngest leaf of the seedling. The stem diameter was measured using a digital caliper, the measurements was taken at the stem base approximately 2 cm above the ground. The number of sheets was conducted by counting the leaves longer than 2 cm.

The leaf area was obtained through linear measurements on leaves, starting from the equation proposed by Wendt (1967) log y = 0.006 + 1.863 log x, where y is leaf area (cm²) exo length of the midrib of the leaf (cm). The leaf area of the plant was measured by the sum of the leaf area of the rolling cottons.

At 120 days after sowing, the crop was harvested and made manually once. After harvesting, the bolls were packed in paper bags with identification and later taken to the Plant Physiology Laboratory, Department of Agriculture. Weighing the bolls of each plant was done manually through delinting for separation of the fiber cores.

Statistical analyses were performed with the aid of Computational Program SISVAR 5.1. The data were analyzed and interpreted from analysis of variance (F test) and by comparison of means by Tukey test, according to Ferreira (2011).

## RESULTS AND DISCUSSION

Statistical analysis revealed statistical significance of salinity levels on leaf number and unit leaf area of BRS Rubi cotton plant, not affecting significantly the plant height, stem diameter and leaf area of the plant. In turn, the quantity of humus do not significantly affect those variables, plant height, stem diameter and plant leaf area, with mean values without significant differences. However, there are significant effects for the variables, number of leaves and leaf unit area. For the unit leaf area variable, the interaction (CEa x H) was statistically significant, indicating that the salinity levels did not behave similarly with the quantities of humus and vice versa.

The number of sheets in relation to levels of salinity in the irrigation water which was adjusted to a quadratic behavior model with a correlation coefficient of 0.99 is shown in Figure 1A. For the increased levels of salt in the irrigation water, there was a reduction in the number of leaves to the level of optimal salinity of 4.5 dS $m^{-1}$ which provided the maximum number of sheets 26. Thereafter, there was a decrease until it reached the level of 6.0 dS $m^{-1}$, which has possibly occurred because of the main consequence of increasing the total concentration of soluble salts in the irrigation water.

The reduction in the growth of plants when they are subjected to salinity is caused due to water deficit caused by the excess soluble salts in the root zone, causing a decline in the turgidity therefore resulting in decreased cell growth and reduction in plant growth (Bai et al., 2008; Khalid and Silva, 2010). This occurs due to the closure of the stomata and consequently lowers $CO_2$ assimilation limiting the photosynthetic processes (Debez et al., 2008; Taarit et al., 2010). Moreover, it may still be caused by energy expenditure which is necessary in the synthesis of organic solutes and the compartmentalization process and regulation of ion transport (Mendonça et al., 2007).

Similar results were obtained by Oliveira et al. (2012a) who observed that the number of sheets has been reduced by increasing the salinity, with a reduction of approximately 4.23 per plant unit in response to increased irrigation water conductivity.

Regarding the effects of earthworm humus amounts applied on the colored cotton plants BRS Rubi (Figure 1B), it is observed that the optimal amount of humus of earthworm was 1.9 kg/plant for the maximum number of 26 sheets. Afterward, there was a decrease in the number of sheets to the extent that it increased the amount of earthworm humus, possibly this decrease was due to nutrient leaching which is responsible for the absence of residual effect of nitrogen in the soil. However, the data adjusted a quadratic polynomial model, with a coefficient determination of 0.99.

Regression equations were fitted to the experimental data of the unit leaf area of cotton BRS Rubi, resulting from the split of the salinity levels interaction in irrigation water set against the amount of earthworm humus that had a quadratic response to the amounts of humus (2 and 3 kg) with determination coefficients of 0.83 and 0.92, respectively (Figure 2).

The reduction in leaf area, issuing new leaves and death and leaf drop occurs due to the effects of salt stress, since these are strategies as a means to reduce water loss (Mahmoud and Mohamed, 2008). In addition, the issuance of new leaves and/or leaf senescence also occurs because these bodies present sensitivity to salinity and reduce the presence of high concentrations of salts. Deleterious effect of salinity on the leaf area was also observed by Medeiros et al. (2011), Vieira et al. (2016) and Lycoskoufis et al. (2012) in tomato. Santos et al. (2016) also observed that the increase in salinity levels affected leaf area, where values decreased by respectively, 7.92 and 9.55% per unit increase in the electrical conductivity of irrigation water.

Statistical analysis revealed significant salinity levels on a number of flower buds, seed number per boll, 100 seed weight, total production, seed production and fiber production. To this end, the quantities of earthworm humus effect were observed only for the number of flower buds. The interaction between salinity levels and quantities of earthworm humus was not statistically significant, indicating that the salinity levels behaved in a similar way with the quantities of humus and vice versa.

For the number of flower buds of colored cotton plants BRS Rubi (Figure 3A), it is observed that as the salinity levels in the irrigation water increased, there was an increase in the number of flower buds. For each unit

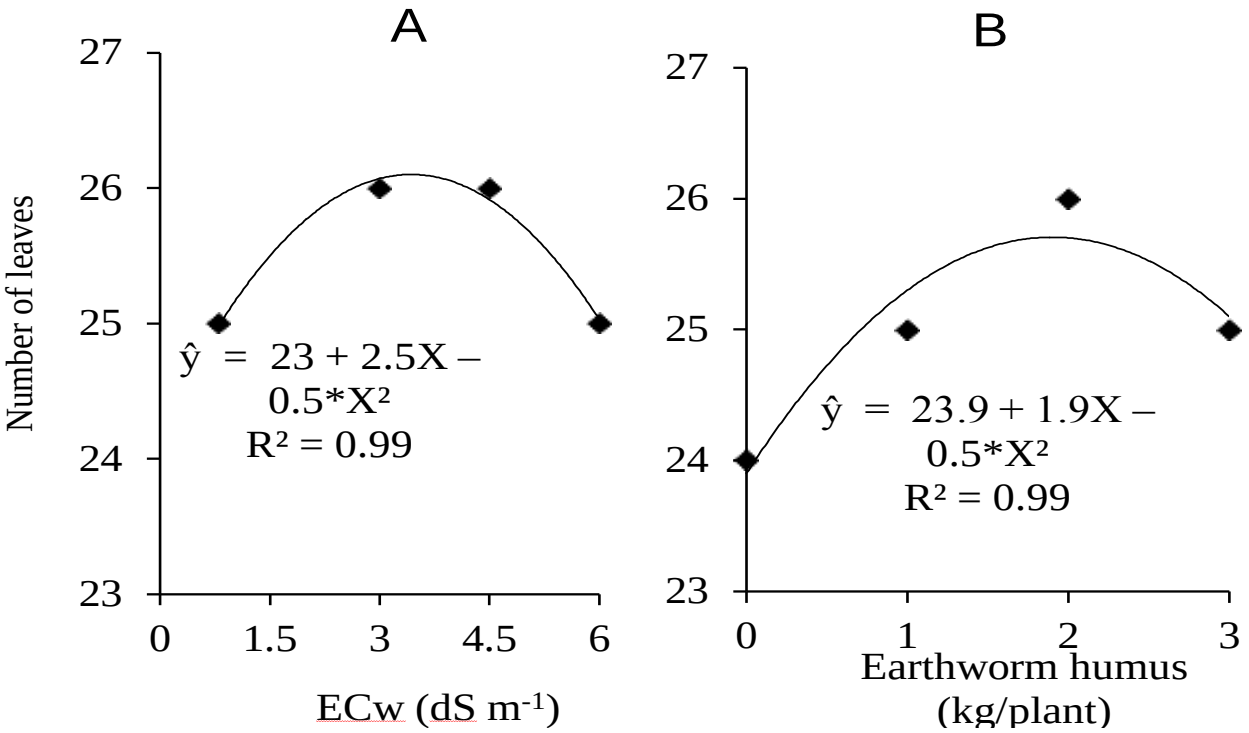


**Figure 1.** Effect of salinity levels (A) and quantities of earthworm humus (B) on the number of leaves on cotton BRS Ruby.

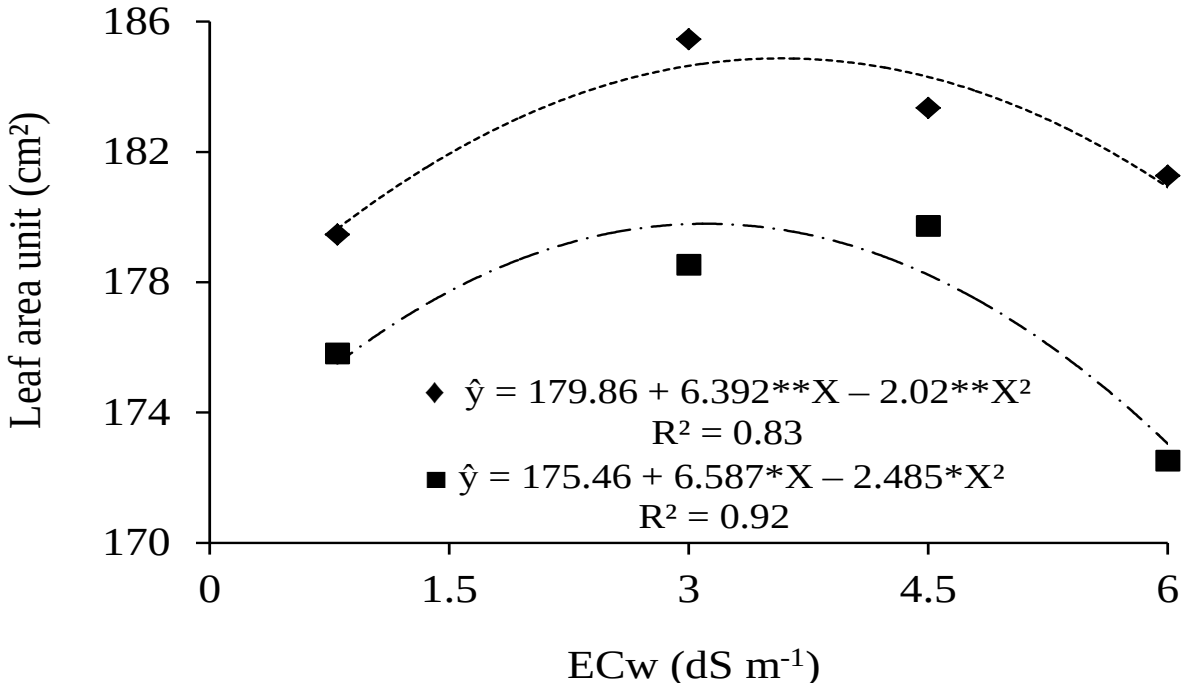


**Figure 2.** Leaf area units of cotton plants BRS Rubi irrigated with saline water, soil ♦ 2 kg/plant and ■ 3 kg/plant of earthworm humus.

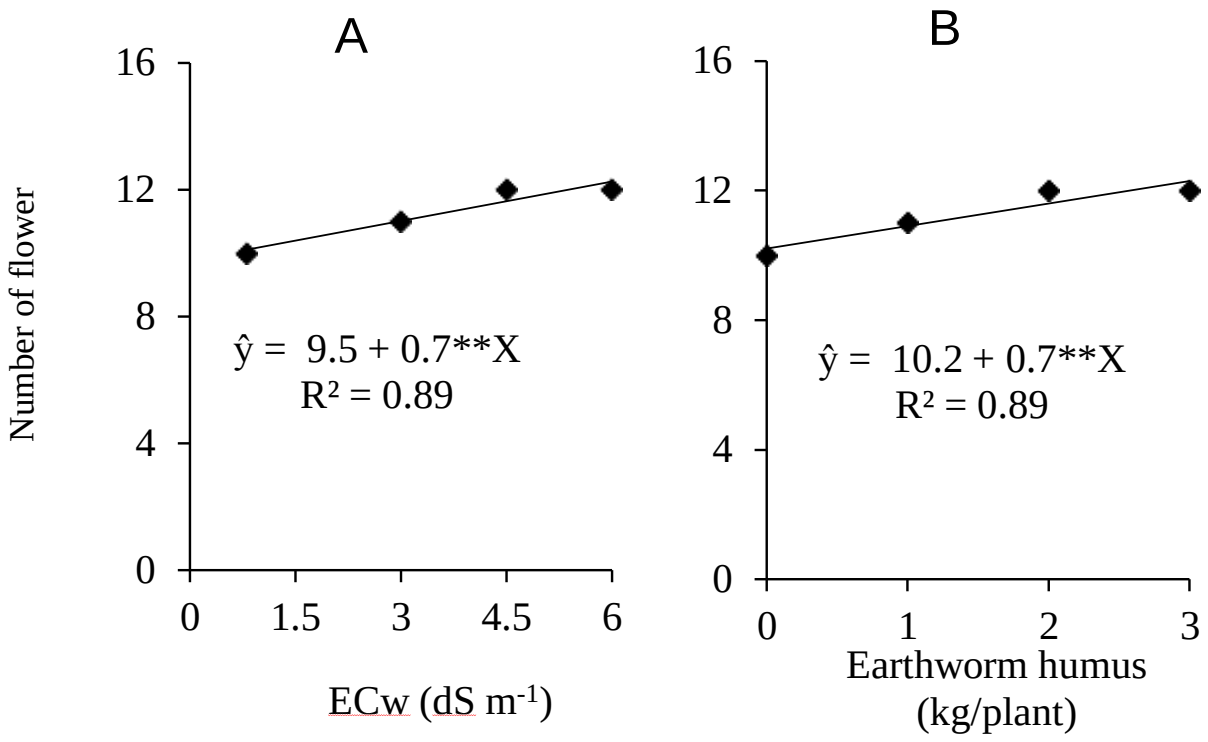


**Figure 3.** Effect of salinity levels (A) and quantities of earthworm humus (B) on the number of flower buds cotton BRS Rubi.

increase in salinity levels, there was an increase of 0.7 in the number of flower buds of BRS Rubi cotton plants.

Regarding the effect of the quantity of earthworm humus on the number of flower buds (Figure 3B), it is observed that there was a linear increase in the number of flower buds on the quantity of humus of earthworm. For each unit increase in the amount of humus, it showed an increase of 0.7 kg/plant in the number of flower buds on cotton plants.

For the number of seeds per colored cotton boll BRS Rubi (Figure 4A), as there is increase in the salinity levels in the irrigation water, there was a decrease in the number of seeds grown cotton plants in greenhouse. For each unit increase in salinity levels, there was a decrease of -0.6 (units) in the number of seeds per boll.

By studying the number of seeds per boll (Figure 4B), it was observed that there was a linear increase in the number of seeds per organic cotton bolls on the quantity of humus California red earthworm. For each unit increase of the applied amount of humus, there was an increase of 0.4 (units) in the number of seeds per bolls on cotton plants.

Choi et al. (2005) studied the growth and production of cotton cultivars under different levels of salinity, and found that the yield in seed of the two cotton cultivars were significantly influenced by the interaction of salinity of irrigation water and genotypes.

The weight of 100 seeds and the total production were adversely affected by salinity levels, and the regression equation adjusted linearly, with decreases in weight of 100 seeds of 60.45% (Figure 5A) and the total production of 58.38% (Figure 5B) per unit increase of salinity levels. It also observed that the maximum seed weight (19.28 g) and the maximum total output (26.94 g) were obtained when the plants were irrigated with water of low salinity (0.8 dS $m^{-1}$).

Oliveira et al. (2012) also observed that the weight of 100 seeds was affected negatively and linearly by salinity of irrigation water, with a reduction of 0.54 g 100 seeds as increased salinity levels, and overall reduction in higher salinity (6.5 dS $m^{-1}$), 28.46% when compared with the results obtained with salinity of 0.5 dS $m^{-1}$. The same authors found out using the weight of 100 seeds, total production was affected by an increase in electrical conductivity of irrigation water, harmful effect was observed from 3.5 dS $m^{-1}$, with a reduction of 52.23%.

Moreover, salt stress causes negative effects on the plant, such as changes in growth and development of the roots, thus interfering with ion water absorption, hindering the development of crops, since a well-developed root

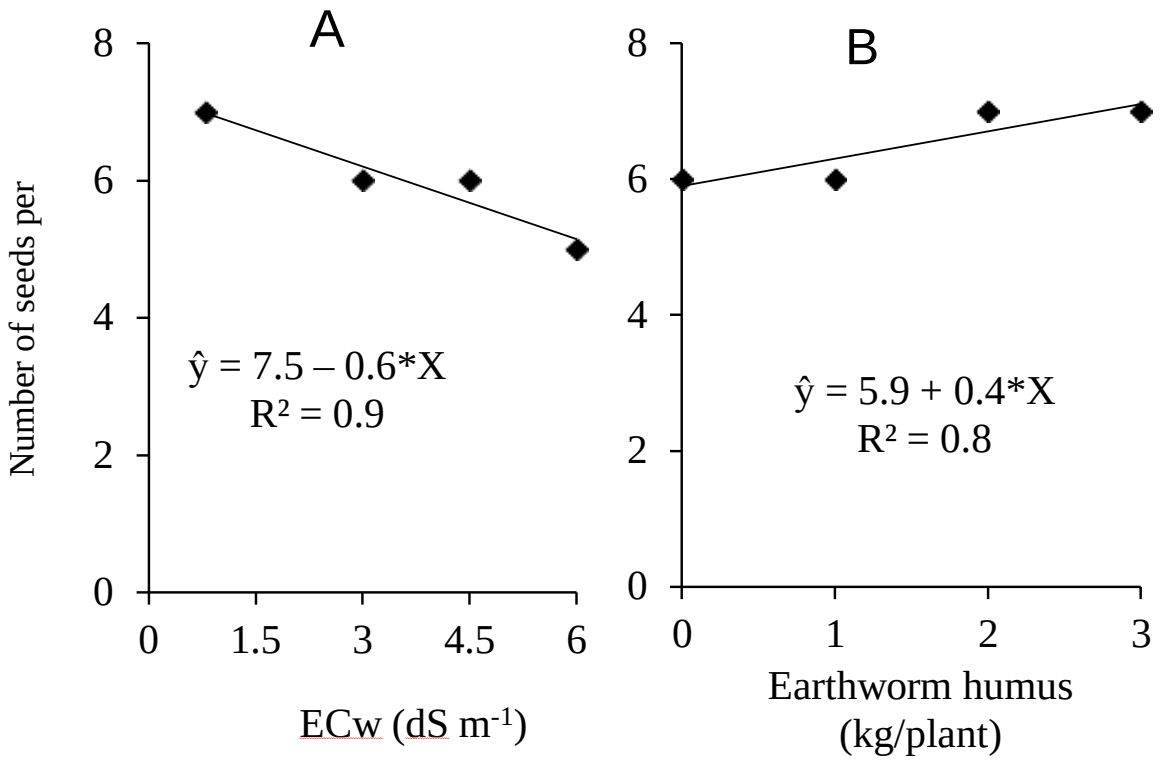


**Figure 4.** Effect of salinity levels (A) and quantities of earthworm humus (B) on the number of seeds per cotton boll BRS Ruby.

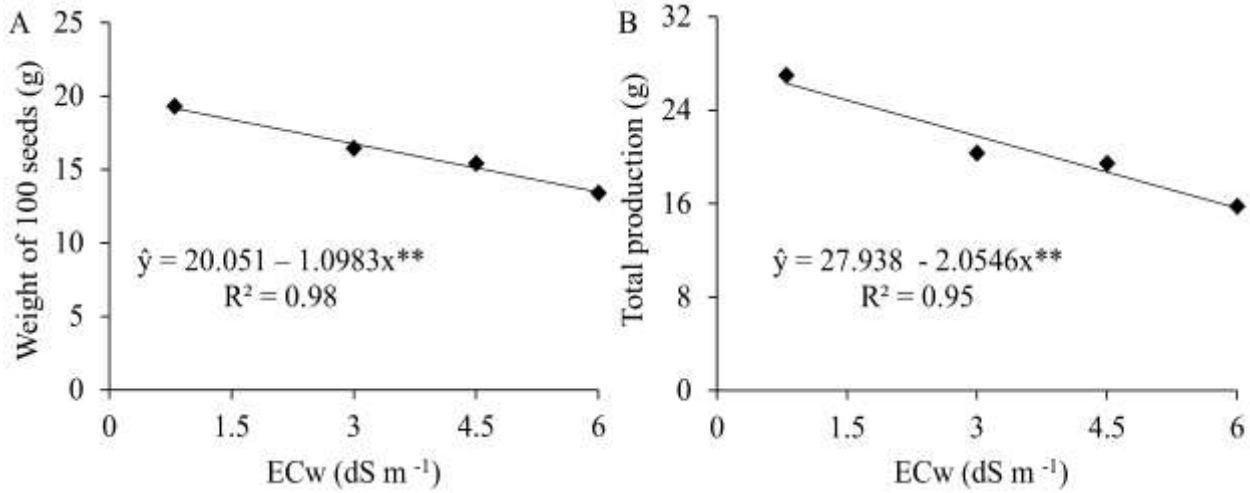


**Figure 5.** Effect of salinity levels on the weight of 100 seeds (A) and total output (B) of cotton, BRS Rubi.

system provides increased absorption area, promotes better conditions to meet the requirements of the plant for water and nutrients, especially in the early days, in seedling stage, when adverse conditions may compromise their survival (Soares et al., 2011). The use of saline waters for irrigation of plants cause various changes in physiological and biochemical functions, many of which result in disturbances in water relations and on changes in the absorption and utilization of essential nutrients to plants, and as a result retard growth

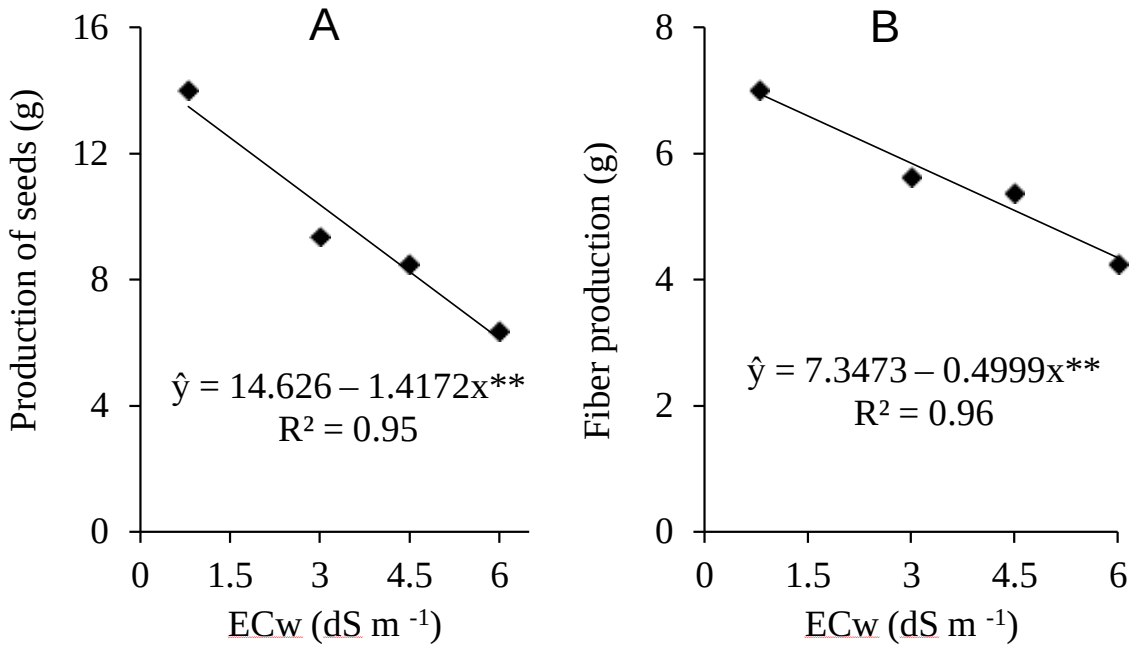


**Figure 6.** Effect of salinity levels on the production of seeds (A) and fiber production (B) cotton, BRS Ruby.

and reduce production (Amorim et al., 2010). It is observed in Figure 6A that the seed production was reduced as the increase in salinity levels. The lower seed yield (6.37 g) was obtained when the plants were irrigated with saline water, 6 dS $m^{-1}$ while higher production was observed at lower salinity (0.8 dS $m^{-1}$),14 g representing a 45.5% loss in grain. For fiber production (Figure 6B), there was a linear reduction as the salinity level increased. Maximum values were observed as 7 g when the plants were irrigated with water of low salinity (0.8 dS $m^{-1}$) and minimum values of 4.25 g irrigation with water of 6 dS $m^{-1}$, representing a 60.71% reduction in fiber production.

Oliveira et al. (2012b) also reported that the production of seed and fiber production were reduced according to the increase in salinity levels, with a significant reduction from the ECw 3.5 dS $m^{-1}$, obtaining values of 8.28 and 12.94 g $plant^{-1}$, respectively. The reduction in the yield of cotton plant can be attributed to the increased conductivity of irrigation water, and in consequence less water absorption by the plants, thus leading to drought stress. Therefore, due to the low capacity of water absorption, the cotton may have a reduction in the production, according to results obtained by Sobrinho et al. (2007).

## Conclusion

The interaction between salinity levels and the quantities of earthworm humus do not affect the growth and production of cotton, while the increase in salinity levels negatively affected cotton crop. Largest earthworm humus quantities provided an increase in cotton production.

## Conflict of Interests

The authors have not declared any conflict of interests.

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African Journal of Agricultural Research

*Full Length Research Paper*

# Method and phenological characterization of the stadiums and phases of the development of castor bean plants

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**With increasing global energy demands, the production of biofuels has been gaining economic importance, with the castor oil plant being one option for the production of biofuel. This study was performed on irrigated cultivation of the castor oil plant, variety IAC 2028, from March to October 2011, in Lavras, Minas Gerais, Brazil, with the following aims: a) to characterise and estimate the sub stages and stages of the crop in days after sowing (DAS) and in growing degree days (GDD) and; b) determine a phenological model of development that describes the vegetative and reproductive behavior of the crop cycle. The experiment was conducted under optimal conditions of irrigation and fertilisation. To determine the phenological model, 15 sub stages and 4 stages of the crop were defined and estimated according to the periodic quantification of variables such as the number of leaves, leaf area, soil cover, and periods of floral initiation, anthesis, bean formation, and maturation by raceme order. We concluded that: a) the sub stages were well characterised and estimated, in particular Vo, A2, M2, and M3, which closed stages I, II, III, and IV with nearly 42, 65, 87, and 100% of the thermal power required by the crop during the lifecycle, respectively; b) the phenological model of the sub stages was determined satisfactorily in GDD; and c) the plant and reproductive sub stages were not completely distinguishable, and the maximum soil cover in M1 indicated that greater productivity could occur with a population increase.**

**Key words:** *Ricinus communis* L., phenological model, phytomass, soil cover fraction, leaf area index.

## INTRODUCTION

The castor bean (*Ricinus communis* L.), is cultivated since the times of the ancient civilizations, the castor bean is a rustic plant, resistant to drought, belonging to the family of Euphorbiaceae, found in many regions of

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the world, including in semi arid areas where it is commercially cultivated between the parallels 40°N and 40°S. The expansion of its cultivations was mainly due to the capacity of adaptation to different environmental conditions and the many applications of use of its main product, the oil (Paixão et al., 2014). This tropical oilseed crop of relevant economic and social importance, currently, global castor seed annual production is around 1.5 million metric tons with four countries (India, China, Brazil, and Mozambique) accounting for 96% of total production. Although the main producing regions are in the tropics, this crop has been grown commercially on large areas in temperate countries such as the United States and the former USSR (Russia and Ukraine). Castor is still being considered for cultivation in regions that experience cool temperatures (10 to 20°C) that prevail in temperate climates and high elevations during the phase of seed filling (Falasca et al., 2012; Severino et al., 2012). From its seeds is extracted an oil of excellent properties, having wide use as industrial input and several applications. The main product which is obtained from the industrialization of castor is the oil, which has industrial uses for the manufacturing of paints, varnishes, soaps, synthetic fibers, plastics, dyes, aniline and lubricants (Torres et al., 2015). Castor oil is unique among vegetable oil because it is the only commercial source of a hydroxylated fatty acid or ricinoleic acid (Serverino et al., 2012). No other commercial vegetable oil produces such a high level of ricinoleic acid. It appears that the level of ricinoleic acid is not significantly influenced by environment. Serverino et al. (2012) also reported that the high content of ricinoliec acid in castor allows the production of high purity derivatives. Currently, castor oil is also used for energy production and in animal diets (Furtado et al., 2012). Studies by (Pertinari et al., 2012), show that the cultivation of this plant can have operating profits that exceed R$ 1800.00 $ha^{-1}$ $yr^{-1}$. Brazil is the third largest producer of castor bean, with a production of 15,800 tons. However, the national productivity of 573 kg $ha^{-1}$ (harvest 2014/2015), is considered low when compared to crop production potential, (CONAB, 2015). In Brazil, and in particular in the southern region of the state of Minas Gerais, information is lacking regarding the interaction of the castor oil plant with local climatic conditions, water requirements and, in particular, there is a lack of studies on the technical and economic factors affecting its production potential. In this region, the average productivity of 1,310 kg ha-1 is higher than the national average, but is lower than the state average productivity of 1,355 kg $ha^{-1}$ (Silva et al., 2010). In this context, this study had the following objectives: a) to characterise and estimate the substages and stages of the castor oil plant based on time and temperature using phenological variables; b) to determine a phenological model of development; and c) to describe the vegetative and reproductive behavior of the crop under the climate conditions of the municipality of Lavras, Minas Gerais, Brazil.

## MATERIALS AND METHODS

In this study, the variety of castor oil plant examined was IAC 2028. The experiment was conducted in a research area of the campus of the Federal University of Lavras (UFLA), Lavras, Minas Gerais, Brazil (latitude: 21° 14′ S, longitude: 45° 00′ W, and altitude: 918.84 m), between 15 March and 20 October 2011, corresponding to a period of 220 days after sowing (DAS). According to the climate classification proposed by Köppen, the climate of the region is Cwa, with an annual average temperature of 19.4°C, annual average relative humidity of 76.2%, and annual precipitation of 1,530 mm. Climatically, the cultivation period coincides with approximately 27.9% of the annual rainfall of the region, and the period from April to September comprises only 16.7%, because March and October are the months at the end and beginning of the rainy season, respectively. The preparation of soil, classified as an oxisol (Red Hapludox) (Embrapa, 2006), consisted of conventional procedures of ploughing and harrowing. To support crop treatments, fertilisation, irrigation, and chemical, physical, and water content analyses were performed at layers of 0 to 0.20, 0.20 to 0.40, and 0.40 to 0.60 m. The cultivation of the castor oil plant in the pre-set period was conducted in three periods: formation of seedlings, initial establishment of the crop in the field, and differentiation of treatments. The formation of crop seedlings was adopted as a way to ensure the population of plants in the plots and to plan the crop lifecycle outside the rainy season of the region, as previously mentioned. The establishment and standardisation of plants was promoted by irrigation and the natural rainfall occurring at the end of the rainy season. The seedlings were grown in a nursery for the period corresponding to the vegetative sub stages of emergence: the formation of cotyledons at 15 DAS (Ve), and seedlings with two complete leaves ready for planting in the field (Vm) on 21 April (38 DAS). At this point, when the seedlings had reached 0.1 m in high and a stem diameter of 0.005 m, the planting and crop establishment step was initiated.

The seedlings in the Vm substage were planted in holes opened manually in furrows and, planting fertilisation and two cover fertilisations were performed immediately after planting. Weed control was performed manually with a hoe, and diseases and pests were not problematic to the point of requiring recommended chemical control. The Vo sub stage, which began at 86 DAS and was characterised by less than 10% soil cover, represents the end of the initial stage of slow growth (I) according to Allen et al. (1998), and it was followed by the F1 substage from 100 DAS through the initial emergence of the first inflorescences. In the first week, 89 mm of water was applied via irrigation through conventional sprinklers in all experimental units. Following this, different experimental units received different amounts of water via irrigation through dripping, initiating the differentiation of steps between treatments at 104 DAS (26 June 2011).

In this work, distinctions were made between primary, secondary, and tertiary orders of racemes, which were defined as having a maximum of 1, 3, and 6 units, respectively, while the lateral racemes had an indefinite number, following Weiss (1983). These were defined as i) primary racemes: situated in the fork between two of the three most vigorous first adjacent branches of the main stem; ii) secondary racemes: situated just above the primary raceme, between two secondary branches; iii) tertiary racemes: situated just above the secondary racemes, between two tertiary branches; and iv) lateral racemes: situated in the lateral branches of the main stem, just below the primary racemes. After Ve, Vm, and Vo, the remaining 12 sub stages were defined by flowering, anthesis, grain formation, and fruit maturation by primary,

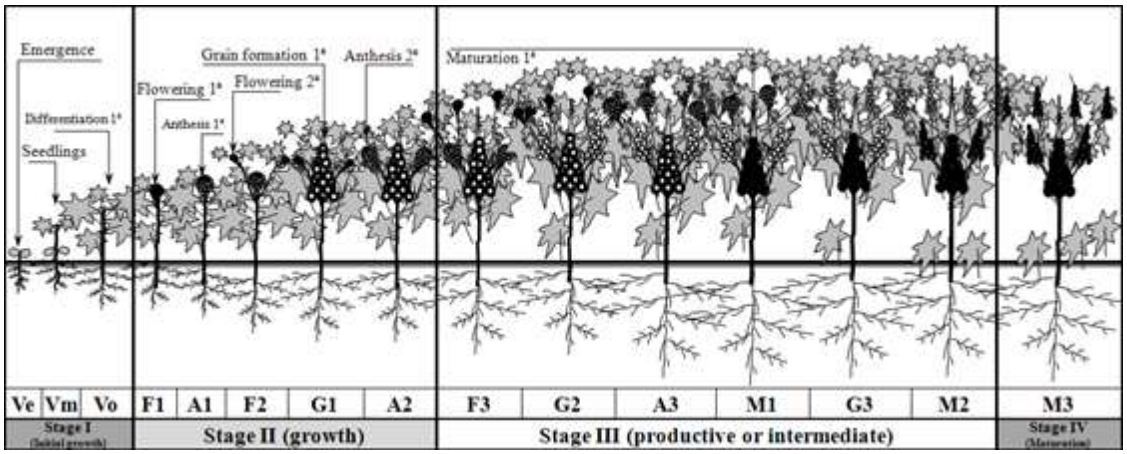


**Figure 1.** Phenology of castor oil plant substages and stages, Lavras, MG, Brazil, 2011.

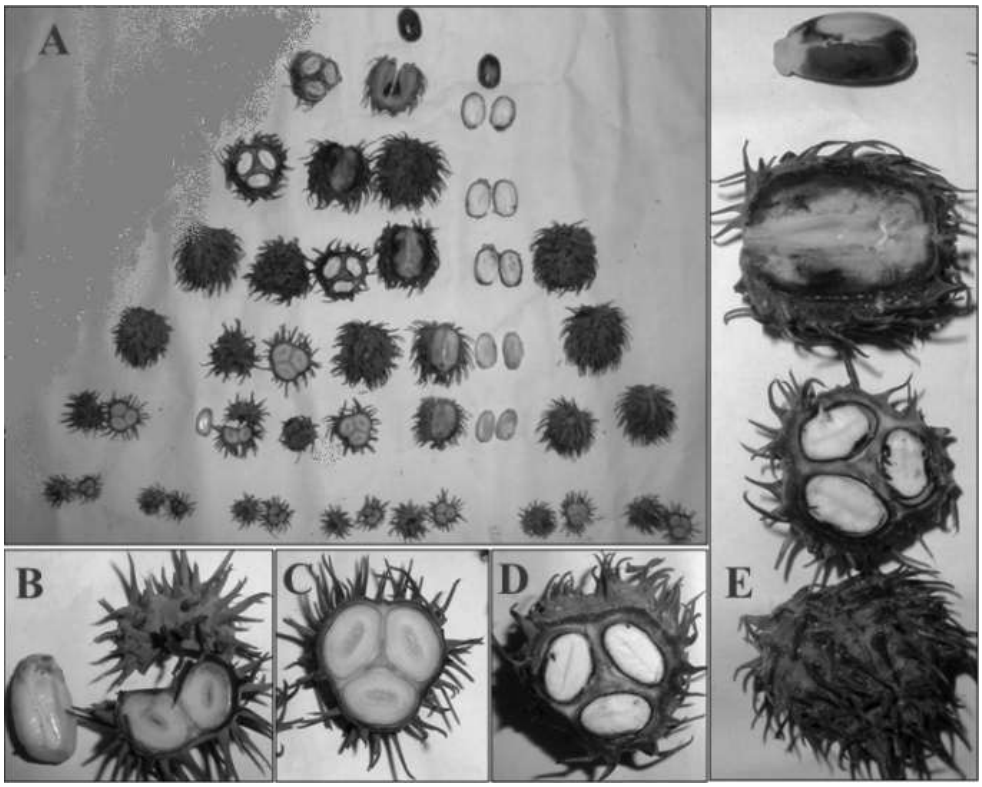


**Figure 2.** Degrees of maturation of the castor oil plant fruits. Pyramid of maturation with immature fruits at the base and completely ripened fruits at the top (A); filled, watery grains with no formed tegument (B and C); filled grains with formed tegument, milky and in physiological maturation (D and E), from Lavras, MG, Brazil, 2011.

secondary, and tertiary orders of racemes: the flowering sub stages, with the initial emergence of the primary (F1), secondary (F2), and tertiary (F3) inflorescences; anthesis sub stages, when more than 50% of examined plants showed emergence of inflorescences (anthesis) in primary (A1), secondary (A2), and tertiary (A3) racemes; the sub stages of maturation, or initial grain filling or equivalent in the primary (G1), secondary (G2), and tertiary (G3) racemes; and maturation substages when, on average, the examined plants showed physiological maturation in more than 70% of the fruits of the primary (M1), secondary (M2), and tertiary (M3) racemes (Figures 1 and 2). The phenological model of the castor oil plant lifecycle, considering the occurrence of the fifteen substages, was sub-divided into stages I, II, III, and IV determined for cultivation according to the criteria and adaptations of Allen et al.

(1998) and Rios et al. (2011), as shown in Figure 1. Stage I, initial slow growth, was initiated with sowing, including Ve and Vm, ending in substage Vo. Stage II, accelerated growth, included the substages F1, A1, F2, G1, and A2, finishing with 80% of maximum soil cover. Stage III, production or intermediate, included substages F3, G2, A3, M1, G3, and M2. Consecutively, stage IV initiated with increasing leaf fall, ending in substage M3. For the purpose of this study, the variables for defining the substages and phenological stages were extracted from an experiment where the interaction between the levels of water application and seasons of irrigation suspension were studied. Since phenological knowledge of crop potential with fertilisation and irrigation under optimal conditions is necessary, for the purpose of this work, we adopted the information extracted from the plots with irrigation at 100% of the quantity of water required to elevate the soil unit to the field capacity during the entire crop lifecycle. Thus, 42 plots were separated for sampling the variables and determining fruit ripening. Each plot comprised 4 rows of 4 plants, with the 4 central plants being examined.

A drip irrigation system was used. The irrigation moment was defined by the matric water potential in soil, measured by 4 tensiometers at 0.30 m depth, reaching an average tension of 26 kPa, equivalent to a depletion fraction of 0.6. The water content at what was considered field capacity corresponded to a tension of 6 kPa and to an effective depth of the roots of 0.4 m (Amaral et al., 2005). The irrigation depth and time were calculated according to Cabello (1996), considering the water content obtained from the water retention curve in the soil, a coefficient of distribution uniformity of 95%, and an efficiency of water application through the system of 90%. Periodic evaluations of the characteristics every week or two weeks were performed to determine the phenological substages of the crop, such as the percentage of ripened fruits in the primary, secondary, and tertiary racemes; dry mass of the aboveground part of the castor oil plant, fraction of dry mass from the stem and leaf petioles; total stem dry mass (except leaf blades and roots), included inflorescences, fruits, and racemes; dry mass of the leaf blade; specific leaf area; and total area per plant. In each evaluation, these variables were obtained by an average of three plants chosen randomly from the plots. The specific leaf area was determined by the ratio between the total leaf area and the dry mass of leaves. The dry mass of the aboveground part of the plant was determined by weighing after drying in a forced air oven, at 65°C until obtaining constant weight, and fruit maturation was estimated by raceme order according to Figure 2 (Fanan et al., 2009). In each plot, all of the leaves on 1 of the 4 studied plants were marked with red string once they emerged. Phenological characteristics were also determined, including the number of leaves per plant, percentage of complete 'adult' leaves (those previously marked by the red string), and number of complete 'new' leaves (recently emerged leaves that had not yet been marked by red string); number of plants with emerged inflorescences; and the presence of primary, secondary, and tertiary racemes. The number of total leaves per plant was the sum of the number of adult leaves and new leaves. The total leaf area per plant was estimated by a photographic method, using a prototype of a new invention attached to the photographic camera, used to measure the unit leaf area obtained directly from destructive samples of leaves extracted from the plant. In field evaluations, the prototype showed identical results to those obtained from a Li-Cor 3000 standard scanner. This measure was obtained from photos processed in the freeware program ImageJ®. The leaf area index (LAI) was calculated as the ratio between the total leaf area and the exposed leaf area occupied by the plant. The soil cover fraction of the crop, a function of the canopy average diameter (D) and spacing (Sf, distance between plants within rows (Sf) × distance between rows (Sp)), was estimated according to Rios et al. (2011), considering the shape as initially a circular area ($D \leq Sp$), then rectangular ($Sp < D \leq Sf$), and then 100% soil cover ($D > Sf$). The growing degree days (GDD) accumulated in each substage were calculated by the residual method considering the maximum and minimum air temperatures (°C) of the nth day after sowing (DAS) and basal temperature of the crop (10°C).

## RESULTS AND DISCUSSION

The Ve and Vm substages began at 15 and 38 DAS. Primary, secondary, and tertiary inflorescences, corresponding to the beginning of sub stages F1, F2, and F3, appeared on average at 100, 124, and 159 DAS, respectively. Primary and secondary inflorescences had 100% emergence at 124 and 159 DAS. For cultivation under normal conditions there is a difference of 10 days between sowing and emergence; therefore, the timing of initial primary, secondary, and tertiary flowering and productive sub stages differed, respectively, by 20, 29, 44 and 30 days from the values of 70, 85, 105, and 180 days after emergence found by Savy Filho et al. (2007). This lifecycle elongation and phenological sub stage elongation likely occurred because of the temperatures and climate conditions of the cultivation period of this study. The substages Vo, F1, A1, A2, and A3 corresponded to, on average, 10% of maximum soil cover (Vo), initial primary raceme emergence and soil cover of 25% (F1), and more than 50% of primary (A1), secondary (A2), and tertiary (A3) raceme anthesis at 86, 100, 120, 149, and 165 DAS, respectively. We also observed that, on average, primary, secondary, and tertiary maturation began at 141, 163, and 192 DAS, respectively, characterising sub stages G1, G2, and G3, respectively, and reached the maximum grain filling, with more than 70% maturation, at 169, 196, and 220 DAS, respectively (Figure 3). Therefore, there were intervals of 28, 33, and 28 days from the beginning to the end of maturation (or a 'period of grain formation of the fruits' of 30 days, on average) and of 69, 72, and 61 days from the emergence of primary, secondary, and tertiary inflorescences, respectively (or 'period of raceme production' being 70 days for primary and secondary racemes and 60 days for tertiary racemes, on average). On average, the intervals between flowering and primary, secondary, and tertiary maturation were similar to the 75, 73, and 73 days, respectively, obtained between the beginning of flowering, at 70, 85, and 105 days after emergence, respectively, and the point of harvesting this cultivar at 145, 158, and 178 days after emergence, respectively, found by Savy Filho et al. (2007) and Fanan et al. (2009). The percentage of maturation exceeded 70% for primary, secondary, and tertiary racemes in the sub stages M1, M2, and M3, respectively, at 165, 196, and 220 DAS, respectively.

During the lifecycle (Figure 4), the number of young leaves, adult leaves, and total leaves, on average, fit a cubic curve, and for adult and total leaves, a slow increase was observed until 86 DAS in stage I (Vo), followed by a sharp increase in stage II until 149 DAS (A2). In stage III at 196 DAS (M2), the numbers of adult

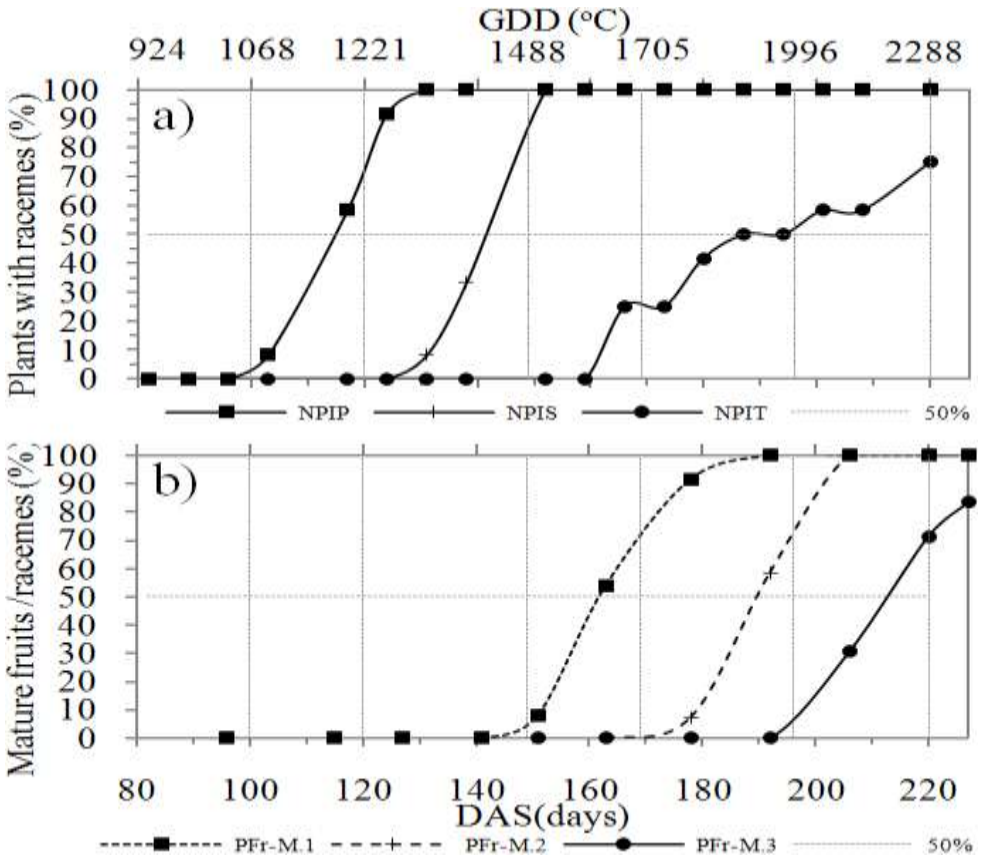


**Figure 3.** Percentage of plants with (a) primary, secondary, and tertiary racemes (NPIP, NPIS, and NPIT, respectively) and percentage of ripened fruits (b) from primary, secondary, and tertiary racemes (PFr.M1, PFr.M2, and PFr.M3, respectively) in days after sowing (DAS) and growing degree days (GDD), Lavras, MG, Brazil, 2011.

and total leaves reached their maximum values at the end of the substage M1 (177 DAS), stabilising in this stage. Subsequently, in the final IV stage, from the M2 substage, we observed a strong decrease in the number of adult leaves and total number of leaves until M3 at the end of the lifecycle (220 DAS). It should be noted that, besides the similar behaviour observed between the adult leaf and total leaf curves, there was a progressive reduction in the difference between their values, of approximately 43, 16, 7, 5 and 0%, in the respective sub stages A1, A2, M1, M2, and M3. This indicated that the initial substages had elevated apical meristematic activity developing young leaves and, for the final substages of the crop, a reduction of that activity, with almost no difference in stage IV, in substages M2 and M3 (Figure 4). The average soil cover fractions in substages Vo, F1, A1, A2, M1, M2, and M3 were, respectively, 10, 25, 45, 70, 84, 82, and 65%, and the maximum soil cover of 85% was at 180 DAS, in the M1 substage. This indicates that, to reach 100% soil cover, an increase in productivity is possible with an increase in the population of plants of up to 17% or a reduction of plant spacing to 1.0 × 0.75 cm. The average specific leaf area can be used to evaluate the photosynthetic efficiency of leaves, deduce their contribution to plant growth, and determine the leaf development for photo assimilates during the sub stages (Magalhães, 1986). According to Benincasa (2003), this initially more elevated index (178 $cm^2$ $g^{-1}$) indicates thinner leaves, with low dry mass and leaf area. This was verified during the initial sub stages of the castor oil plant until sub stage A1 (Figure 5), and stabilised from there until M3 at the end of the lifecycle (average 142 $cm^2$ $g^{-1}$), with a slight fall after sub stage M2 (average 133 $cm^2$ $g^{-1}$), possibly associated with leaf senescence or the emergence and/or permanence of thicker leaves with low leaf expansion and with nutrient redistribution. This stabilisation of specific leaf area between A1 and M2 may be explained by the increase in the number of leaves and/or the expansion of leaf area, reflecting a higher photosynthetic capacity. The LAI expresses the photosynthetic capacity of the crop, varying, among other factors, with plant density, which affects both the

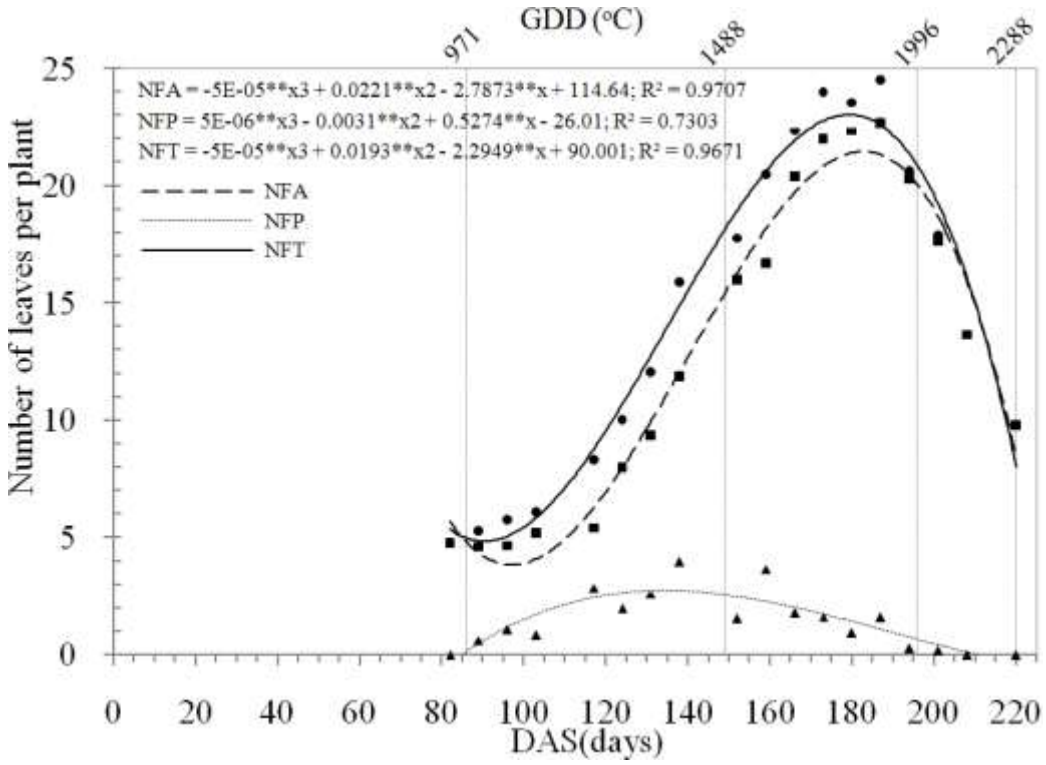


**Figure 4.** Number of adult leaves, (NFA) newly emerged leaves (NFP), and total leaves per plant (NFT), in days after sowing (DAS) and growing degree days (GDD). ** indicates significant coefficients by a t test at 1% probability. Lavras, MG, Brazil, 2011.

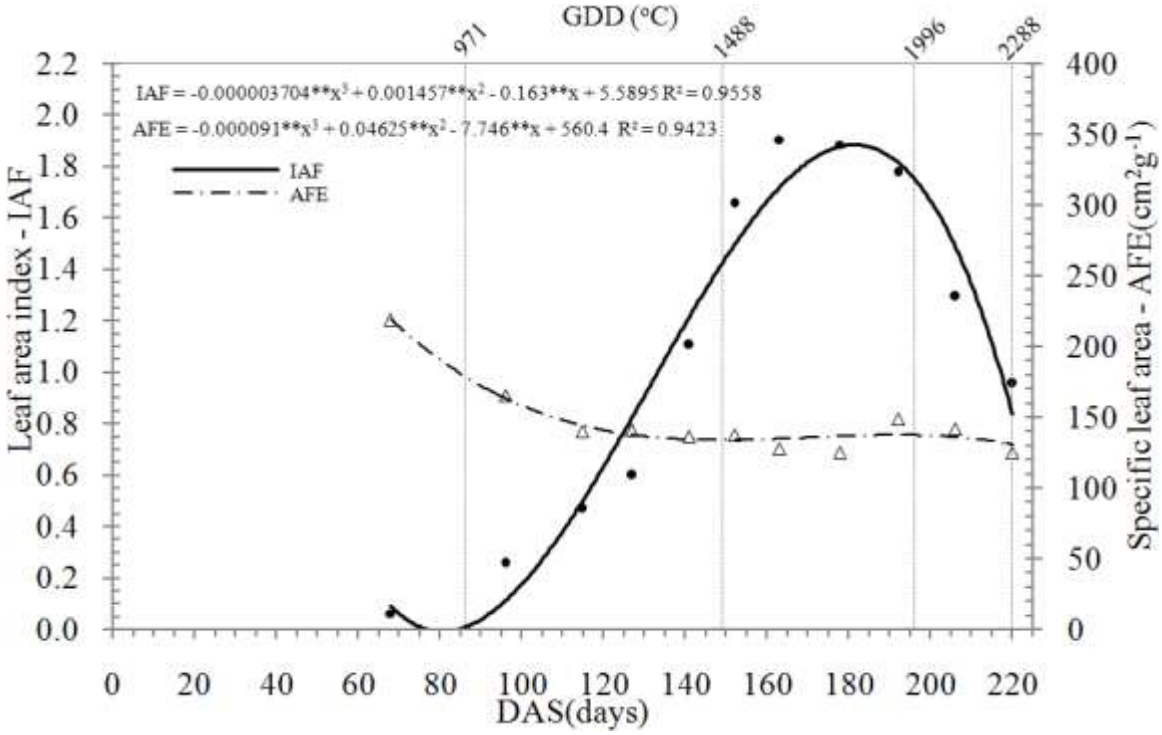


**Figure 5.** Leaf area index (LAI) and specific leaf area (SLA), in days after sowing (DAS) and growing degree days (GDD). ** indicates significant coefficients by a t test at 1% probability. Lavras, MG, Brazil, 2011.

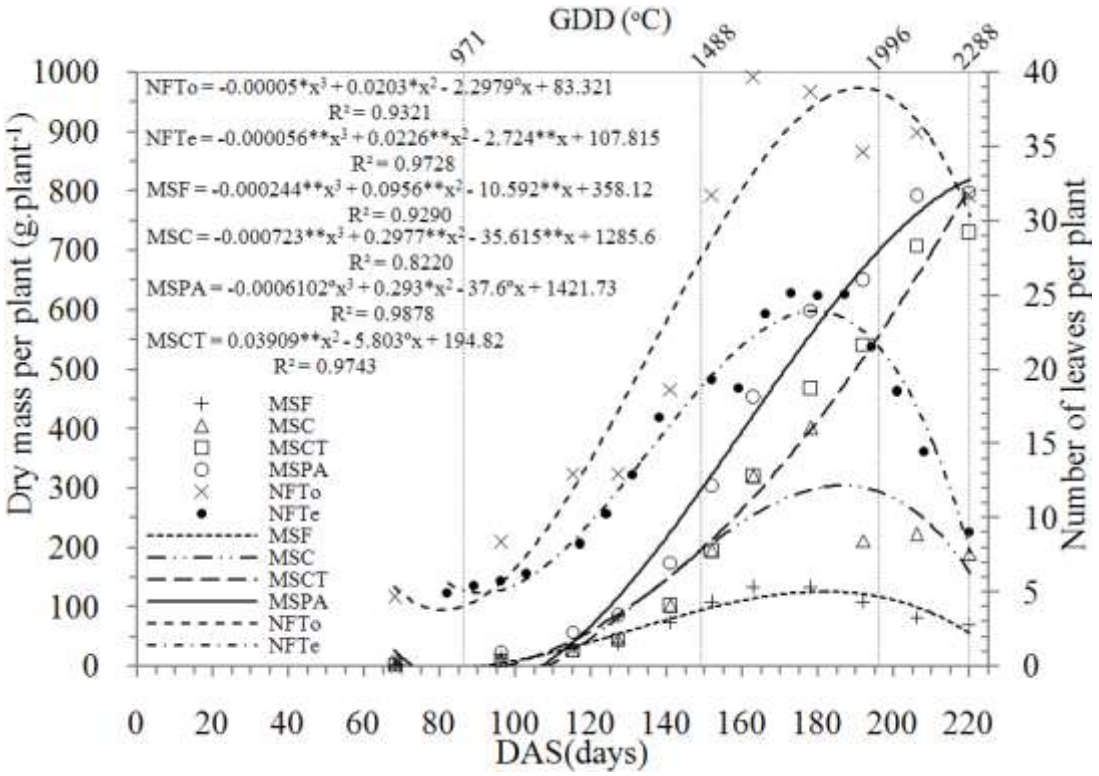


**Figure 6.** Total number of leaves observed (NFTo) and estimated (NFTe) per plant; aboveground dry mass per plant (MSPA), dry mass of the stems (MSC), aboveground dry mass except leaf blades (MSCT), and dry mass of leaf blades (MSF), in days after sowing (DAS) and growing degree days (GDD). °, *, and ** indicate, respectively, significant coefficients by a t test at 10, 5, and 1% probability, respectively. Lavras, MG, Brazil, 2011.

maximum LAI reached by the crop, and the time elapsed from emergence until growth stabilisation and, consequently, the absorption of incident solar radiation. Kotz (2012) obtained a maximum LAI of less than 3 from the castor oil plant 'IAC 2028' at harvesting, close to that obtained in a soya crop (Heiffig et al., 2006). According to Beltrão et al. (2007), the LAI of the castor oil plant crop under non-irrigated conditions varies between 2 and 4. In the first sub stages, the leaf area is small and, as a consequence, the LAI is low, causing large losses in radiation usage, with more light directly reaching exposed soil (Andriolo, 1999; Heiffig, 2002). With the development of the crop and, consequently, of leaf area, the interception of solar radiation reaches a maximum, but still without shading problems for the lower leaves. From this point onwards, when self-shading starts to occur, the lower leaves are in deficit in terms of photosynthesis, and the increase in dry matter and leaf area tends to stabilise.

In stage I, approximately 40% through the lifecycle, but near the beginning of sub stage F1 (at 100 DAS, stage II; Figure 6), growth was very slow, with, on average, approximately 5 leaves per plant, aboveground dry mass of 25 g plant$^{-1}$, soil cover fraction of 10%, LAI of 0.1, and specific leaf area of 157 cm$^2$ g$^{-1}$. This initial logarithmic or exponential trend in growth, according to Beltrão et al. (2005), may be associated with the adaptation of seedlings to the field environment, to climate conditions of cultivation outside the recommended season, and morph physiological characteristics, specifically directed in this stage to root establishment. However, it should be emphasized that, in those conditions, considering among other factors, the spacing used and the critical period of prevention of interference from invasive plants, it is possible that castor oil plants could be grown with other annual crops of high economic value and an earlier lifecycle, such as corn, beans, sunflowers, or cucurbits. In stage II, the linear stage, we observed a sharp vegetative growth of the crop until close to the end of that stage in sub stage A2, and the beginning of stage III, at 149 DAS. In stage III, growth occurred with decreasing yields until the end of sub stage M1, at 177 DAS, stabilising until reaching estimated maximum values of 38 leaves per plant, SLA of 1.9, leaf dry mass of 125 g plant$^{-1}$, and maximum stem dry mass of 300 g plant$^{-1}$ (MSC), with the end of that stage in sub stage M2, at 196 DAS, when the beginning of the end of stage IV occurred, with a strong decrease in growth, until the end of the lifecycle in sub stage M3, at 220 DAS (Figure 6).

**Table 1.** Phenological characterisation of the castor oil plant, iac 2028, substages, stages, and average values of growth during the crop lifecycle, lavras, mg, brazil, 2011*

| E | Abr. | Substage (E) | Description | Stages | Description | DAS (days) | GDD (°C) | LAI (adm) | fc (%) | NFT (und) |
|---|---|---|---|---|---|---|---|---|---|---|
| e1 | Ve | Emergence | Cotyledons | I | Vegetative | 15 | 208 | … | ... | … |
| e2 | Vm | Seedlings | 2 leaves, h=15, d = 0.5 cm | I | Vegetative | 38 | 507 | 0.01 | 1 | 2 |
| e3 | Vo | Initial of F1 | LAI = 0.1 or 5 leaves | I | Vegetative | 86 | 971 | 0.10 | 10 | 5 |
| e4 | F1 | Flowering 1 | Flowering raceme 1 | II | Repr./Veg. | 100 | 1068 | 0.10 | 25 | 6 |
| e5 | A1 | Anthesis 1 | Anthesis raceme 1 | II | Repr./Veg. | 120 | 1221 | 0.61 | 45 | 10 |
| e6 | F2 | Flowering 2 | Flowering raceme 2 | II | Repr./Veg. | 124 | 1251 | 0.75 | 48 | 11 |
| e7 | G1 | Grain formation 1 | Grain formation raceme 1 | II | Repr./Veg. | 141 | 1408 | 1.25 | 63 | 16 |
| e8 | A2 | Anthesis 2 | Anthesis raceme 2 | II | M./R./Veg. | 149 | 1488 | 1.40 | 70 | 19 |
| e9 | F3 | Flowering 3 | Flowering raceme 3 | III | M./R./Veg. | 159 | 1593 | 1.65 | 76 | 21 |
| e10 | G2 | Grain formation 2 | Grain formation raceme 2 | III | M./R./Veg. | 163 | 1634 | 1.75 | 78 | 23 |
| e11 | A3 | Anthesis 3 | Anthesis raceme 3 | III | M./R./Veg. | 165 | 1657 | 1.80 | 80 | 23 |
| e12 | M1 | Maturation 1 | Maturation raceme 1 | III | M./R./Veg. | 169 | 1705 | 1.82 | 81 | 24 |
| e13 | G3 | Grain formation 3 | Grain formation raceme 3 | III | M./R./Veg. | 192 | 1950 | 1.80 | 84 | 22 |
| e14 | M2 | Maturation 2 | Maturation raceme 2 | III | M./R./Veg. | 196 | 1996 | 1.72 | 82 | 21 |
| e15 | M3 | Maturation 3 | Maturation raceme 3 | IV | Mat.+Repr. | 220 | 2288 | 0.81 | 65 | 8 |

*1 – stages according to Allen et al. (1998); Abr.: Abbreviation of substages; GDD: growing degree days and DAS: days after sowing, both cumulative data; LAI: leaf area index observed; fc: soil cover fraction; and NTF: estimated total number of leaves.

From the beginning to the end of stage IV, there were declines from 38 to 30 leaves per plant; from 1.9 to 0.8 LAI; from 125 to 57 g plant$^{-1}$ leaf dry mass; and from 300 to 160 g plant$^{-1}$ stem dry mass. However, at the end of stage IV, the maximum values were reached for total aboveground non-leaf dry matter (810 g plant$^{-1}$) and total aboveground mass (834 g plant$^{-1}$; Figure 6). It is worth mentioning the underestimation of estimated total leaves compared to observed total leaves, which probably occurred due to the counting process in the field, with small complete leaves not counted and eventually hidden inside the canopy of the plant. Stages I, II, III, and IV had durations of 86, 63, 47, and 24 days, respectively, corresponding to 39%, 29%, 21%, and 11% of the crop lifecycle, respectively. The first 2 stages were longer, similar to what was reported by Rios et al. (2011). However, these results are unlike those described by Allen et al. (1998) of a lifecycle of 180 days with the last 2 stages of greater duration (III and IV), with percentages in stages I, II, III, and IV of 14, 22, 36, and 28%, respectively. It is worth mentioning that the vegetative growth and reproductive sub stages of those stages were not completely distinguishable, since both continued to occur simultaneously and indefinitely, depending on water availability, soil fertility, favourable weather conditions, and control of pests and diseases. Table 1 summarises the sub stages and stages of the crop, the respective average values of LAI, soil cover, and total leaves per plant previously discussed, in DAS or in GDD accumulated during the lifecycle. Stages I, II, III, and IV had thermal durations of 971, 518, 508, and 291 GDD, respectively (2288 GDD in total), corresponding to 42, 23, 22, and 13% of the lifecycle, respectively.

Taking into account the thermal energy required by the sub stages and stages obtained from Table 1, these thermal requirements may be better described and visualised by a 2-axis graph called a phenological model. In this model, the proportions are specific for each crop or variety, functioning as a 'fingerprint', except for the occurrence of deviations from the experimental data (Figure 7). In the phenological model, the axes are the growth axis (X) and total development of the crop (Y) according to the gold ratio ($X/Y = \phi \approx 1.618$), with the measure of the X axis corresponding to the thermal time of the crop lifecycle (2288 GDD, with scale X:2288 GDD), and the measure of the Y axis corresponding to the sum of 'n steps' equally weighted (n/N) by the total number (N = 15) of sub stages of the crop (Ve, Vm, ...), and each horizontal interval between steps corresponding to the duration of the sub stages, which also compose the stages I, II, III and IV. Therefore, in order to obtain the total thermal energy from the end of stage I in sub stage Vo, for example, it is enough to measure the X axis and xo in Vo, and deduce the measures Y and yo in Vo by the proportions $X/Y = \phi$ and $n/N = yo/Y$, with n and N the numbers, respectively, of sub stages until I(n) and of the crop lifecycle (N). It is worth mentioning the closeness of the sub stages A1 and F2; F3, G2, and A3; and G3 and M2 (Figure 7), which could have been grouped to simplify the phenological model, resulting, in this case, in twelve sub stages, as described by Moshkin as cited by Beltrão (2002) and Silva et al. (2008).

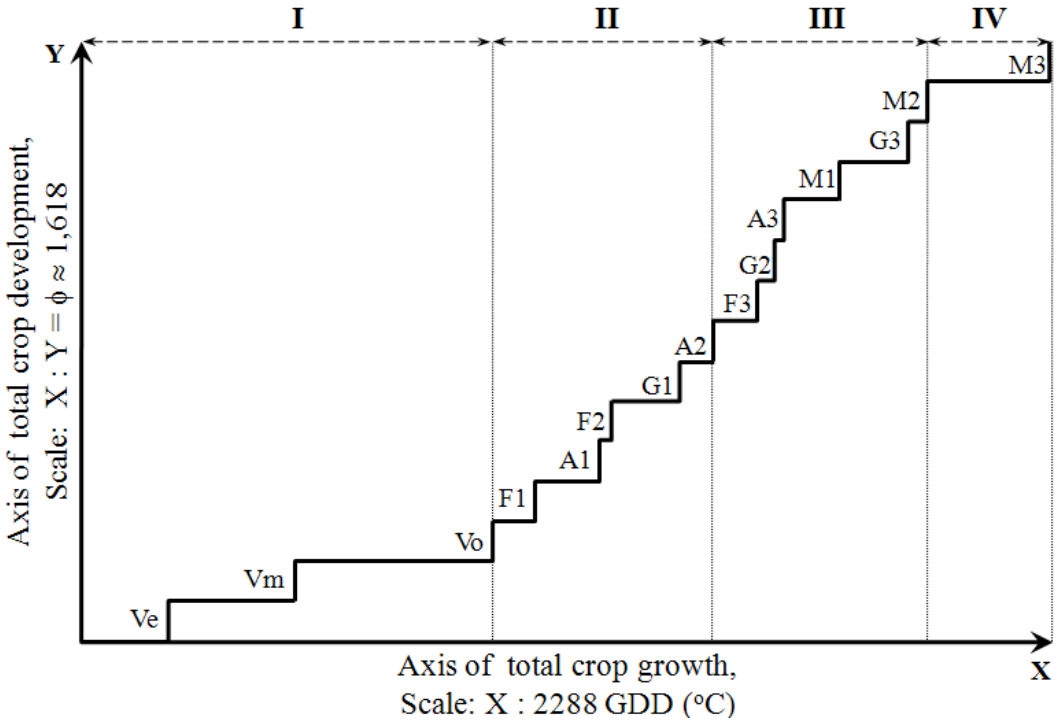


**Figure 7.** Phenological model of the castor oil plant, IAC 2028, represented with the axes X and Y in the golden ratio (X/Y = Φ ≈ 1.618), where 'n steps'. are substages (Ve, Vm,...) of measures equally weighted by the total number (n/N) in Y, and the intervals between them the sub-stages or stages (I, II, III, and IV) in X. Lavras, MG, Brazil, 2011.

## Conclusions

1) The sub stages were well characterised and estimated, in particular the primary flowering, secondary anthesis, and secondary and tertiary maturation, which finalised the respective stages I, II, III, and IV, with approximately 42, 65, 87, and 100%, respectively, of the thermal energy required during the crop lifecycle.
2) The phenological model, a function of the required thermal energy, number of sub stages, golden ratio, and other criteria, was determined satisfactorily to describe and quantify the sub stages.
3) The crop lifecycle and stages showed longer periods than those described in the literature; however, in the initial stage, cultivation with other crops is possible, considering the spacing used and the critical period for preventing invasive plants. The vegetative and reproductive sub stages occurred simultaneously and indefinitely under favourable conditions, and the maximum soil cover fraction occurred in primary maturation, indicating the potential for an increase in productivity with an increase in the population.

## Conflict of Interests

The authors have not declared any conflict of interests.

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# Determinants of profit in sustainable forest management in the Brazilian Amazon

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**The sustainable management of tropical forests has been a great concern and challenge for the forest sector in the Brazilian Amazon. This study aimed to better understand sustainable forest management in the Brazilian Amazon focusing on two research questions: (a) Is sustainable tropical timber production financially viable? (b) What are the profit determinants under sustainable forest management? In this research, we assessed information of all approved forest management plans in 2011 in the Sinop region, located in the eastern Brazilian Amazon. Sequentially, we selected and tested using econometric tools these variables: Profit, managed area, volume of timber, number of managed species and timber price. Our results show that the average profit is of US$ 1,003.00 per hectare in sustainably managed forests and we observed that the variables volume of timber per hectare and timber price explain the profit of this forest activity presenting the best fit econometric results.**

**Key words:** Economic analysis, forest management, timber production, tropical forest.

## INTRODUCTION

Sustainable Forest Management (SFM) had become an important research topic in the 1990's since the definition of its principles in 1992 at the United Nations Conference on Environment and Development in Rio de Janeiro (Wang, 2007). Sustainable management is a broadly accepted terminology that defines forest management according to the principles of sustainable development and, more specifically, it involves balancing social, economic and environmental values related to forest resources and taking these values into consideration for future generations (Canova, 2012).

The SFM has been an alternative forest use to guarantee continuous long-term tropical timber production and those forest related environmental services. However, the SFM needs to be properly certified to assure to the consumers that forest products meet the environmental criteria. As a result, certification efforts have been fostered as a means of improving sustainability in tropical forest management (Agrawal, 2008).

Selective logging activities in the Amazon rainforest have been carried out in predatory ways. The adoption of practices for SFM is crucial and only a few logging

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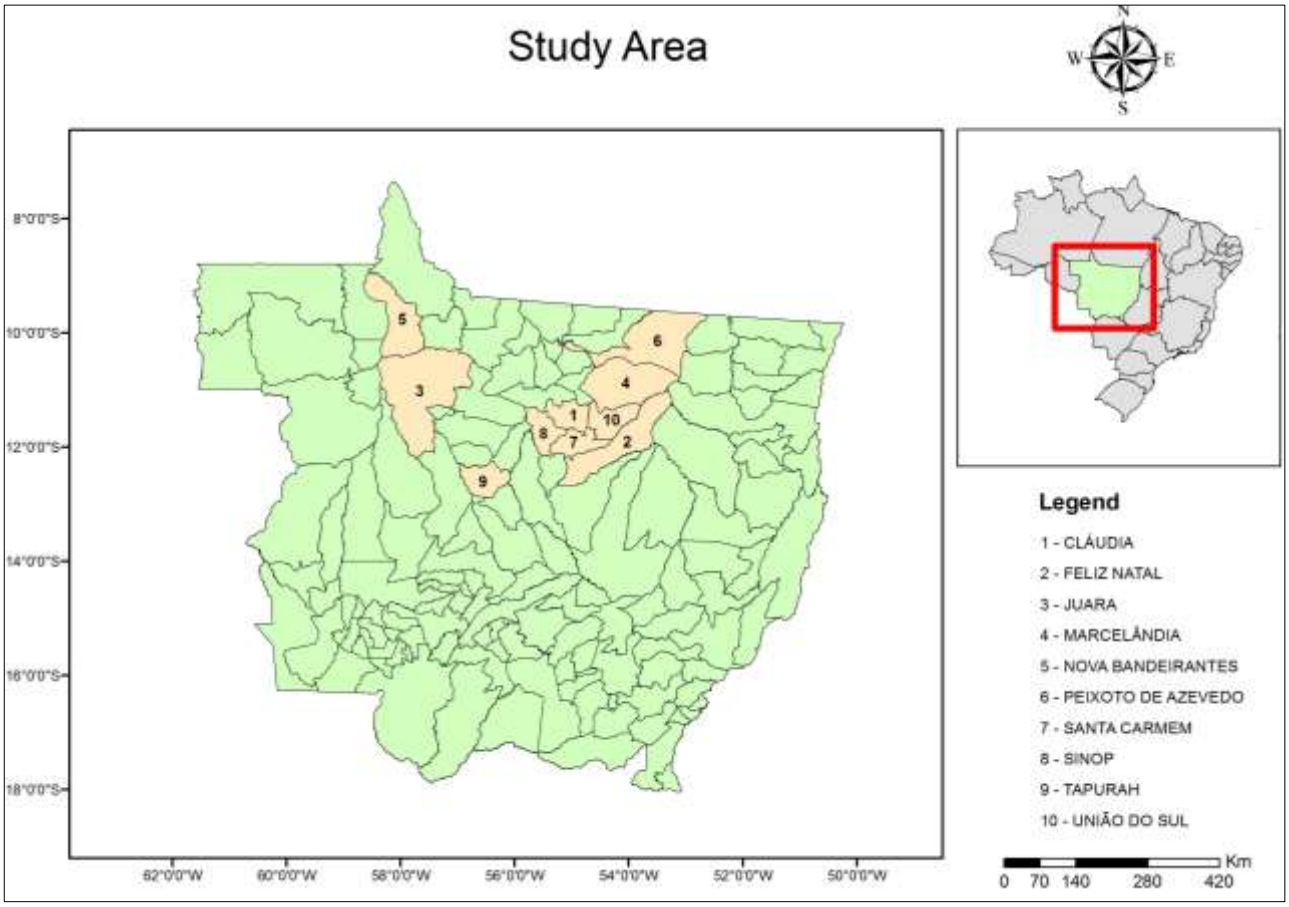


**Figure 1.** Study area.

companies are practicing sustainable logging in the Brazilian Amazon. Nevertheless, it is important to promote sustainable logging in the Amazon Basin to assure long-term supplies of environmental goods and services, including climate regulation, regional and global precipitation regulation, conservation of biodiversity and supply of timber and non-timber products (Banerjee et al., 2009).

Although SFM is economically viable, some authors argue its financial viability since there are many barriers to its large-scale application in the Amazon region. Examples of such barriers are: The incipient spread of management techniques among forest users; the higher profitability of agriculture in the short term if compared to forest management; and the lack of efficient control of non-sustainable logging, making it profitable in the short term (Canova, 2012). Overcoming those regional barriers would require implementation of appropriated forest policy that includes a public land occupation plan in the region, an efficient logging control, further economic incentives to forest management and technical assistance forest landowners (Barreto, 1998; Richards, 2000).

Finally, legal regulation itself has not been enough to improve the adoption of SFM. Due to the lack of financial support for SMF, uncertainties of the harvesting costs in sustainably managed forests by forest entrepreneurs, lack of information on management techniques and its economic impacts on forest sustainability. Therefore, a more detailed economic analysis is needed to assess costs and benefits of that type of forest management and to provide information to the development of the sustainably management of tropical forests (Barreto, 1998).

This study aimed to better understand the sustainable timber production in the Brazilian Amazon. More specifically, we assessed financial viability of certified selective logging and its profit determinants.

## MATERIALS AND METHODS

This research was conducted in the Sinop region, state of Mato Grosso, located in the southern Brazilian Amazon (Figure 1). This region is considered an important timber center in the Brazilian Amazon. The dataset was created by assessed information of all approved forest management plans in 2011 formally approved by

the Mato Grosso Environmental State Agency for the highlighted municipalities in Figure 1. The study area included the municipalities of Sinop (a timber center), Claudia, Feliz Natal, Juara, Marcelândia, Nova Bandeirante, Peixoto de Azevedo, Santa Carmem, Tapurah, and União do Sul, located within the Amazon state of Mato Grosso.

The independent studied variables used in this analysis included selectively logged area, the number of harvested tree species, harvested timber volume ($m^3ha^{-1}$), total logging income, and total logging cost.

The data used in this study were acquired by interviewing the 26 owners of forest management plans during the 7th edition of "Promadeira" Meeting at the International Fair of Wood, Furniture, Machine and Equipment of the Forest-Based Sector. That international event lasted for one week during October 2011 in Sinop city, state of Mato Grosso, Brazil.

**Economic analysis**

The profit per hectare (Π) of timber extraction under sustainable forest management was estimated using Equation 1.

$$\Pi_t = TR_t - TC_t \quad (1)$$

Where: $\Pi_t$ = profit of sustainable timber extraction in dollar per hectare (US$/ha) in the period t; $TR_t$ = total revenue of timber production in dollar per hectare (US$/ha) in the period t; $TC_t$ = total cost of sustainable timber production in dollar per hectare (US$/ha) in the period t.

Equation 2 as function of forest management profit presented in a log-linear form as the following was used to estimate the profit determinants:

$$\ln\Pi_t = \beta_0 + \beta_1 \ln MA_t + \beta_2 \ln NS_t + \beta_3 \ln TV_t + \beta_4 \ln TP_t + \varepsilon \quad (2)$$

Where $MA_t$ = the forest managed area (hectares) in the period t; $NS_t$ = the number of harvested tree species per hectare in the period t; $TV_t$ = the harvested timber volume ($m^3$ per hectare) in the period t; $TP_t$ = the average price of harvested timber in each forest management project in the period t (US$/$m^3$), and ε = the stochastic error.

All variables used in this analysis were transformed (logarithm) as an empirical convenience. Subsequently, the Marshallian elasticities were directly estimated for the used variables in this analysis. Further details is given in previous study (Wooldridge, 2009).

The variable (*MA*) is the forest-managed area (hectares) in 2010. The variable (*NS*) is the number of harvested tree species. The variable (*TV*) is the total harvested timber volume ($m^3ha^{-1}$). The total harvested timber volume was limited to 30 $m^3ha^{-1}$ due to the Brazil´s environmental regulation (Brazil, 2006). The variable (*TP*) is the timber price estimated based on the gross income of timber production divided by the total harvested timber volume. Therefore, the variable *TP* is the average price of all harvested tree species (US$/$m^3$).

The variable (*MA)* was intended to estimate effects of the scale production (forest managed area / total profits of the forest activity). Based on it, our first hypothesis is that the size of the managed forests is directly related to its profit. It is expected that the forest owners will harvest larger areas to increase their profits as the legal regulations limit the timber volume per unit area. The forest-managed area (*MA*) was used to estimate area of harvested forests in the study period.

The number of tree species (*NS*) estimates timber diversity. Commonly, loggers choose the most valuable tree species. The dominant tree species in the study area are: Cedrinho (*Erisma uncinatum* warmi), Cambará (*Vochysia* sp.), Itaúba (*Mezilaurus itauba*), Angelim-pedra (*Dinizia excelsa* Ducke), Peroba (*Aspidosperma polyneuron*). Other species that appear with a lower frequency are Peroba-cupiúba (*Goupia glabra* Aublet), Amescla (*Trattinnickia burseraefolia*), Garapeira (*Apuleia leiocarpa*) and Tauari (*Couratari oblongifolia* Ducke).

The harvested volume per hectare (*TV*) indicates forest productivity and financial profits by logging activities. That productivity, however, is currently limited to 30 $m^3ha^{-1}$ by the environmental regulation applied for the Brazilian Amazon. The forest management plans only are approved if they respect this limitation although even with the government surveillance a small group of timber producers sometimes harvester more than the limit or without authorization and then try to sell it in an illegal market but in this study we gone work only with the official data.

The timber price (*TP*) is directly related to the financial profit resulted of selective logging. In this case, a close relationship between timber price and profit supports the hypothesis that loggers tend to choose the most valuable tree species for logging.

Equation 1 was arranged by its variable order and rank conditions as suggested by Pindyck and Rubinfeld (1991). Additionally, its coefficients were estimated by the ordinary least squares method.

The econometric efficiency of the models was tested by applying the Snedecor's F statistic. This test intended to assess the dependent variable effects on the independent variables. The Student's t statistic was applied to assess the individual effects of the independent variables on the dependent variables; Durbin-Watson's d statistic was applied to verify the presence or not of autocorrelation in the stochastic terms.

Equation 2 was estimated by the ordinary least squares method (OLS). The OLS assumption is that the coefficients should be expressed as β1, β2, β3 and β4> 0 for one-tailed t test. Multicollinearity was assessed by estimating the indicator of Variance Inflation Factor (VIF). Heteroscedasticity was assessed by using Durbin-Watson's d test, the BPG test (heteroscedasticity), and the RESET test (specification) at a 0.10 significance level. Gujarati (2011) suggested the d test in case of indecision zones and the Geary test may be applied (1970).

## RESULTS

Table 1 shows further details of the studied variables. The harvested timber profit for sustainably managed forests varies from US$ 178.00 to 2,257.00 per hectare and an average of US$ 1,003.00 per hectare (estimated values in October 2010). Based on it, we observed that forest management for timber production is a profitable business and this large profit variation can be explained by the type of the species and the extract volume by hectare in the property because a property with a high valued species and a high volume extraction will be much more profitable than a property with low valued species and low volume extraction that is a natural fact and can occur.

Regarding the remaining studied variables (*MA and NS*), it is worth highlighting the extensive forest areas management demand which has an average value of 1,221.00 ha, and the low number of species extracted by hectare.

By comparing forest and soybean profits, it was observed that soybean profitability in 2011 varied from US$ 137.94 to 1,009.18 in the municipality of Sorriso, state of Mato Grosso (Lima, 2012). The profit responses

**Table 1.** Characteristics of the studied variables.

| Variables | Area (ha) | N° of extracted species/ha | Volume extracted $m^3$/ha | Total revenue US$/ha | Total cost US$/ha | Profit US$/ha |
|---|---|---|---|---|---|---|
| Average | 1221 | 2 | 27 | 2540 | 1538 | 1003 |
| Minimum | 300 | 1 | 20 | 891 | 594 | 178 |
| Maximum | 4000 | 8 | 30 | 3861 | 1877 | 2257 |

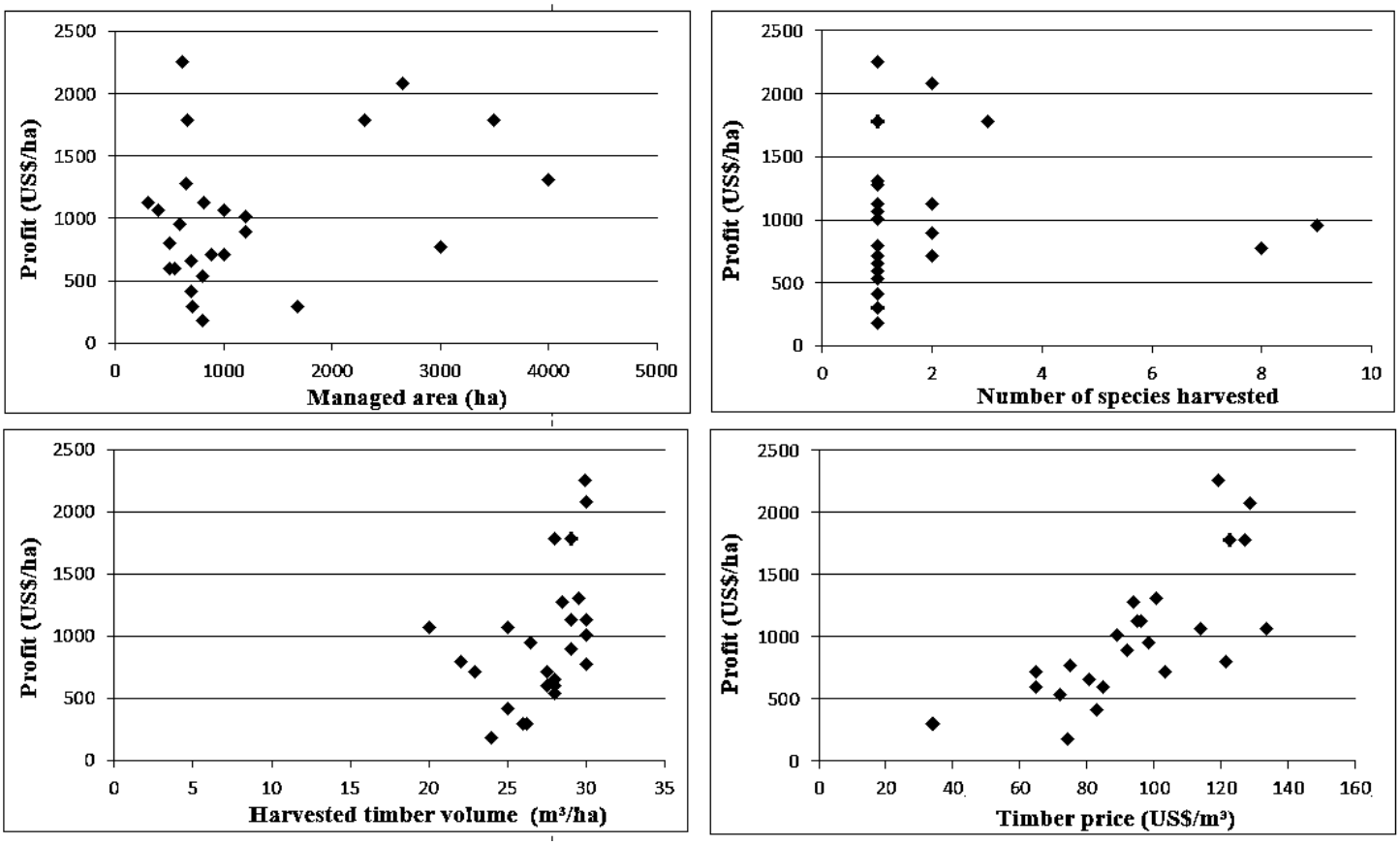


**Figure 2.** Evolution of profit (Π) in relation to the managed area (*MA*), the number of extracted species (*NS*), volume of timber extracted (*TV*) and timber price (*TP*).

of timber production from sustainably managed forests to the area managed, the number of tree species, and timber price are shown in Figure 2. Based on correlation analysis results, the timber profit of managed forests showed moderate relationship with the harvested volume (0.45), and strongly relationship with the timber price (0.78).

The multiple regression model is used to obtain the elasticities of the dependent variables and estimated the profits. The Table 2 shows the statistical results of the estimated linear relationship among the studies independent and dependent variables in this analysis. The result of this analysis showed that the multiple regression model can significantly explain 78% of the dependent variable (timber profit of sustainably managed forests) at α = 0.01 (probability of 99%). In the above specification, all variables present the signs expected according to the theory. More specifically, the harvested volume per hectare (*VE*) and timber price (*P*) were significantly different at α = 0.05. The managed forest area (*AM*) and number of tree species per hectare (*NE*) were not different at α = 0.05, which means that these two explanatory variables have no significant effects on timber profit. However, they are important explanatory variables for the comprehension of the profit function and for that reason they remained in the model. Finally, the

**Table 2.** Estimated parameters of the sustainable forest management profit equation.

| Constant | lnMA | lnNS | lnTV | lnTP | $R^2$ | F | d | Rho |
|---|---|---|---|---|---|---|---|---|
| -8.10 | 0.07 | -0.03 | 2.40 | 1.39 | 0.78 | 18.38 | 1.08 | 0.22 |
| (-3.64) | (0.69) | (-0.24) | (3.72) | (7.52) | 0.32[I] | | | |

Student's *t* statistic in parentheses; Rho = 1st order autocorrelation of residuals; [I]standard error of residuals; d = Durbin-Watson *d* statistic.

results of Durbin-Watson *d* statistic test indicates (1.08) residual independence of the dataset at 95% probability level.

## DISCUSSION

The timber profit of sustainably managed forests is highly dependent on the timber harvested volume per hectare (*VE*) and its effects were estimated as following: if the timber harvested volume increases by 10%, the timber profit will increase by 24%. The estimated elasticity was 2.4 for the timber harvested volume, which indicates an elastic response.

The hypothesis that Timber price (*P*) significantly and positively affects the timber profit of sustainably managed forests cannot be rejected, and its effects were estimated as following: If the timber price increases by 10%, the timber profit will increase by 13.9%. These results indicate that by increasing timber demand and prices, there will increase profits of forest owners or managers. By increasing tropical timber prices, it is expected that forest management will be even more profitable.

Another hypothesis assumed that by increasing number of tree species should directly increase timber profit. Contrarily, we observed a negative effect from number of tree species on timber profits, most likely due to the relatively small number of trees that are commercially valuables for selective logging in the Amazon region. One of the critical aspects of forest management in the Brazilian Amazon, which is the small market of commercial species. Therefore, by harvesting a larger number of tree species, there will increase the overall forest management costs and those additional harvested tree species would not be appropriately accepted and valued by the market and consumers.

Timber harvesting under SFM can be financially viable, even in the absence of payments for other services, in some tropical forests. These will include those where high-value tropical timber species are present in sufficient numbers to support a low-volume, high-value timber harvesting regime; a certification system will be necessary as a tool for maintaining access to high-value markets. They may also include forests with sufficient natural species' homogeneity (the timber of which is sufficiently distinct to command a 'loyal' market niche), or where a high density of currently 'lesser known' species prove to be marketable enough, to allow the development of a strong value added sector (Leslie, 2002).

A complementary strategy is to implement SFM in areas where such high-value timbers are not found in sufficient quantities, or where forests are not sufficiently homogenous, supplemented by direct payments for other, global, goods and services. With such payments, SFM that includes timber production could well become financially viable in some regions (Leslie, 2002).

Fortini and Carter (2014) studied the viability of small-scale forest management activity in the estuary of the city of Magazão, located in the state of Amapá. The authors estimated a positive profit and an internal rate varying from 22 to 84% for the production types studied on their analysis.

By conducting an economic analysis of low-impact logging in the Abunã region, located in state of Rondônia, Sartori (2012) observed that a forest area of 560 ha, with an initial investment of approximately US$ 409,294.00, it is possible to reach a profit return between US$ 76,743.00 and 153,485.00 up to the third year of forest activities. By applying Monte Carlo Simulation, Sartori estimated an internal returning rate greater than 13.8% at 90% of probability.

In the Brazilian Amazon, according to the Forest Code, until 2012, 50 to 80% of all landholdings had to be conserved as forest, where only sustainable management of timber and non-timber forest products is allowed. According to official data, at least 40 million hectares of forests are held by smallholders and communities and could potentially be managed through sustainable forest management (SFM). In some states, the existing demand for timber may only be met in the future with an expansion of CFM or small-scale SFM (Piketty, 2015).

The tools available for encouraging SFM begin with policy and regulations that support those who are practicing forest management. They also include inventories, monitoring, forest management certification, stakeholder involvement and forest management plans. Where there is a clear understanding of the ecological circumstances of the forests being managed an appropriate regulatory framework can establish the enabling conditions for SFM (Macdicken, 2015).

Finally, we understand that the harvested volume must be limited by the current Brazilian environmental law. Also, any change in harvesting timber volume per hectare must observed growth rates (volume) of each tree species. That would guarantee that forest production is

actually economic, environmental, and social sustainable.

## Conclusions

The estimated equation shows great potential to contribute and support sustainable forest management in the Amazon region, and also provides a rational use of tropical species and bases for the creation of policies to the forest management. Based on this research results, we observed the economic viability of the forest management activities in that region if forest plans are well prepared and properly carried out.

Sustainable forest management requires, however, competitive market prices of tropical timber worldwide. Moreover, the harvested timber volume per hectare substantially contributes to explain profit behavior in sustainable forest management.

We estimated that the elasticity of the harvested timber volume was around of 2.40, which indicates an elastic response. A similar result was observed for the timber price variable (~1.39). We argue that elasticity may decrease as legal restrictions are established and enforced in tropical timber commercialization. It is likely due to the small size of fraction of demand. The timber price components and harvested volume per hectare affected profit behavior in this analysis. As a result, we observed a profit positive feedback to the timber price and harvested volume.

Like in other countries, one of the main challenges in Brazil is to increase the competitiveness and attractiveness of SFM compared with other land uses. Understanding monetary costs and benefits thus plays a central role in developing equitable benefit sharing arrangements and assessing whether the net gains from timber harvesting are sufficient to encourage a community's long-term commitment to SFM for commercial purposes (Piketty, 2015).

Forest communities and small farmers in the Brazilian Amazon are important actors in the sustainable management of the forest, as they control nearly 60% of public forests in the Brazilian Amazon. Promoting sustainable forest management and incorporating it in agrarian production systems will play a key role in the fight against deforestation in the near future (Sist, 2014).

## Conflict of Interests

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Soil micronutrients status assessment, mapping and spatial distribution of Damboya, Kedida Gamela and Kecha Bira Districts, Kambata Tambaro zone, Southern Ethiopia

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**Micronutrients are important for crop growth, production and their deficiency and toxicity affect crop yield. However, the up dated information about their status and spatial distribution in Ethiopian soils is scarce. Therefore, fertilizer recommendation for crops in the country has until recently focused on nitrogen and phosphorus macronutrients only. But many studies have revealed the deficiency of some micronutrients in soils of different parts of Ethiopia. To narrow this gap, this study was conducted in Kedida Gamela, Kecha Bira and Damboya districts of Kambata Tambaro (KT) Zone, Southern Ethiopia, through assessing and mapping the status and spatial distribution of micronutrients. The micronutrients were extracted by using Mehlich-III multi-nutrient extraction method and their concentrations were measured by using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). The fertility maps and predication were prepared by co-Kriging method using Arc map 10.0 tools and the status of Melich-III extractable iron (Fe), Zinc (Zn), boron (B), copper (Cu) and molybdenum (Mo) were indicated on the map. The extracted Fe ranged from 50.04 to 209.72 mg/kg, 60.08 to 240 mg/kg and 46.84 to 412.23 mg/kg in Kedida Gamela, Kecha Bira and Damboya districts soils, respectively. Zn ranged from 1.3 to 28 mg/kg, 0.9 to 47 mg/kg and 1.0 to 39 mg/kg for Kedida Gamela, Kecha Bira and Damboya districts, respectively. The calculated manganese activity index (MnAI) indicated that Mn is in excess and even toxic. B ranged from 0.02 to 1.83 mg/kg, 0.4 to 1.44 mg/kg and 0.06 to 2.03 mg/kg in soils of Kedida Gamela, Kecha Bira and Damboya woredas, respectively indicating that most of soils were deficient in B. Cu ranged from 0.6 to 2.9 mg/kg, 0.5-1.44 mg/kg and 0.8 to 3.4 mg/kg in Kedida Gamela, Kecha Bira and Damboya districts indicating its status fall between low and optimum category. Mo ranged from 2.21 to 18.71 mg/kg in the soils of the study area indicating that all soils were sufficient in Mo content. The means of all micronutrients except B showed significant differences among districts and showed moderate spatial dependences. The range of semivariogram for all studied micronutrients was greater than the average sampling distance indicating that it was adequate enough to catch spatial variability of them. In order to strengthen this result, plant sample analysis and calibration of micronutrients with plant response are recommended.**

**Key words:** Boron, Copper, Iron, Kriging, Manganese, Mehlich-III, Molybdenum, Zinc and spatial dependency.

## INTRODUCTION

Elements like Fe, Mn, Zn, Cu, B, Mo, Co and Cl are known to be essential for plant growth and are required in small quantities; hence, they are called micronutrients or tracer elements. In plants, they are important for protein and auxin production (Zn), as constituent of cytochrome oxidase (Cu), photosynthesis (Fe), carbon assimilation and nitrogen metabolism (Mn) (Arokiyaraje et al., 2011). They are all harmful when the available forms are present in the soil in large quantities and indiscriminate use of micronutrients is not advisable because of the small amounts needed and their interaction with other nutrients (Yadav and Meena, 2009). Maximizing agricultural production needs, among others, a balanced use of micronutrients (Patel and Singh, 2009). The deficiency or the excess presence of micronutrients such as Fe, Mn, Zn and Cu may produce synergetic and antagonistic effects in plants. As a result, either deficiency or excess (toxicity) of micronutrients results in abnormal growth, which sometimes cause complete crop failure. Thus, micronutrient deficiency and toxicity can reduce plant yield (Tisdale et al., 1995). Besides, grain and flower formation does not take place in severe deficiency (Nazif et al., 2006). Therefore, correcting micronutrient deficiencies through balanced fertilization promotes yield of crops (Wondwosen and Sheleme, 2011). In view of the above considerations, knowledge of the status and the spatial distribution of micronutrients become very important to revise the fertilizer package to boost crop productivity.

Due to misunderstanding or feelings that remarkable response doesn't occur from micronutrients application, until recently, it was concluded that micronutrients deficiency was not serious problem in Ethiopian soils (Desta Beyene, 1982). However, most recent studies confirmed that certain soil micronutrients were deficient in soils of Ethiopia which limit crop productivity. The deficiencies of Mo, Cu, and Zn are mainly reported on Ethiopian Nitisols (Teklu et al*.,* 2003). Also, Yifru Abebe and Mesifn Kebede (2013) reported the deficiency of Fe and Zn in the majority of soil samples collected from the Vertisols of central Ethiopia.

Many studies revealed that judicious use of nitrogenous and phosphatic fertilizers in the intensive cropping system may cause the quick depletion of micronutrients in soils (Katyal and Randhawal, 1983). In addition to this, the availability of micronutrient to plant growth is highly dependent on some soil factors such as organic matter content, adsorptive surface, soil pH, lime content, soil texture, topography and nutrient interactions in the soil (Nazif et al., 2006; Eyob Tilahun et al., 2015). Thus, mapping the status and spatial variability of soil micronutrients in agricultural soils and adjusting their availability to plants through balanced fertilization is expected to increase crop productivity. Ethiopian Soil Information System (EthioSIS) being implemented by the Agricultural Transformation Agency (ATA) and Ministry of Agriculture and Natural Resources (MoANR) is currently pursuing complete soil fertility assessment by applying GIS and geostatisticcal tools with recent methods and models to come up with solid, evidence-based and targeted balanced fertilizer recommendations and other management interventions for agricultural land soils of Ethiopia. Currently, the fertility mapping and fertilizer recommendation work for the majority of the country's agricultural land has been completed. The project also gave attention for micronutrients by noting that crop production cannot be boosted by application of only DAP and urea that have been distributed to farmers until 2013 (EthioSIS, 2014, 2015).

Thus attention should be given to access to up-to-date data about status and spatial variation of micronutrients to ensure sustainable agricultural productivity. However, in Southern Nations, Nationalities and Peoples Regional State (SNNPRS) of Ethiopia information on micronutrient status at district level is scarce. Therefore, in this study, an attempt has been made to assess the status of Fe, Mn, Zn, B and Cu in the agricultural soils of Kedida Gamela, Kecha Bira and Damboya districts of KT zone of SNNPRS Ethiopia to map their status and spatial distribution and verify which areas require micronutrient fertilizer(s).

## MATERIALS AND METHODS

### Study areas

### Location

This study was conducted in Kedida Gamela, Kechabira, and Damboya woredas of Kambata and Tembaro (KT) Zone in 2014. Kembata and Tembaro (KT) is one of the zones of the Southern Nations, Nationalities and Peoples Regional State (SNNPRS) in Ethiopia. Geographically, the study area is situated at 7.12 to 7.42° latitude and 37.44 to 38° longitudes (Figure 1) and is situated approximately 250 km south-west of Addis Ababa. The whole KT zone is situated between 1500 and 3500 m above sea level (masl), and the topography is characterized by steep slope at the foot of Anbericho, Dato and Ketta mountains and valley sides to Holagaba Zato peasant association. However, the study areas situated 1689 and 2637 m.a.s.l.

### Land use and vegetation

Mixed crop-livestock system is the main land use system in the

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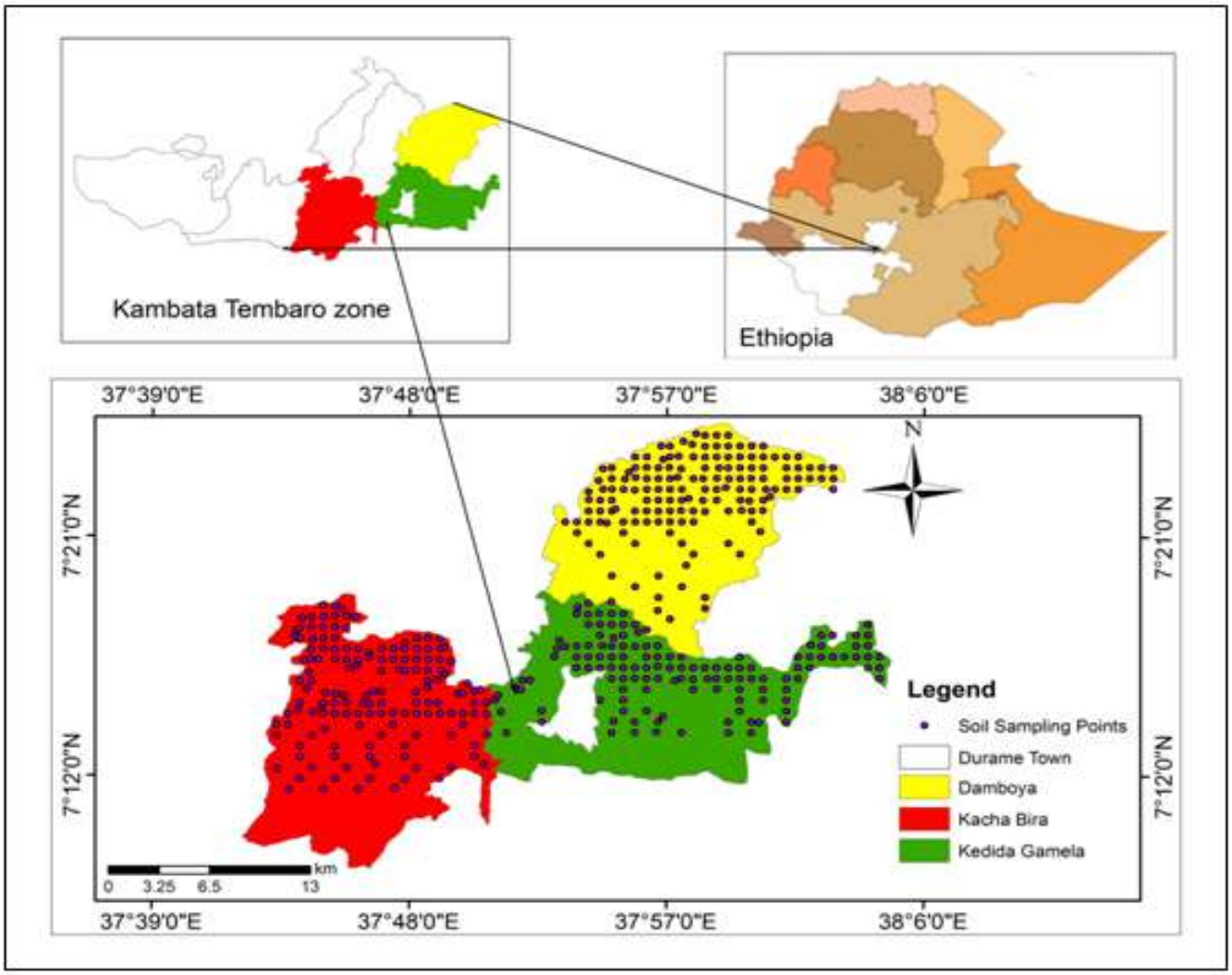


**Figure 1.** Map of the study area.

study Woredas. The major food crops grown in the study area are maize (*Zea mays* L, teff (*Eragrostic tef* Zucc. Trotter), wheat (*Triticum aestivum*), enset (*Ensete ventricosum*), Barley *Hordeum vulagare* L.) sorghum (*Sorghum biocolor* L., potato (*Salantum tuberosum*), faba beans, (*Vicia faba)*, field peas (*Pisum stivum)*, millet *(Elevsine coracana)* and other cereal crops and vegetables. Coffee (*coffee Arabic*) and chat (*Catha edulis Forsk*) are the dominant non-food cash crops in the study areas. Agriculture is entirely rainfed.

There are few types of natural vegetation such as *Juniperus procera, Olea africana and Hajenia abyssinica* in the grazing and arable land. However, in most of the study area eucalyptus trees are dominant trees and are replacing the natural forests. In addition, there are different grass species such as Elephant grasses covering the ground on the grazing lands especially in strongly sloping plain and hilly slope areas.

**Soil sample collection**

Surface soil samples were collected through gridded survey method over agricultural land of the study Woredas where sampling points were spaced 1 km from each other. Predefined sampling plots were identified and used to take samples following the field guideline. Samples were taken from locations having similar soil types, topography and similar land use history or land utilization type (LUT). Soil sampling was carried out by using GPS by navigating those Pre-defined points. Plot and center of the sub-plots were determined by letting the GPS which is fitted on Samsung tablet average position for at least 3 to 5 min.

Based on the topography and soil variability, in total, 156, 149 and 155 composite soil samples were collected from the agricultural soils of Kedida Gamela , Kecha Bira and Damboya woredas, respectively. The soil sampling depth was 0-20 cm for annual crops and 0-50 cm for perennial crops. For all soil types, 10 subsamples were collected within 15 m distance between and among each sub-sampling points in a circle method and composited. For each main sampling point, about 1 kg of representative composite soil sample was collected and logged into properly labeled plastic sample bag.

Soil samples were not taken from restricted areas such as animal dung accumulation places, poorly drained and any other places that cannot give representative soil samples. During soil sampling, data of spatial information (latitude and longitude), topography, slope, site, land use type, crop type, local soil name, sampling depth, soil

**Table 1.** Critical levels used for classifying soil fertility parameters analysis result (EthioSIS team analysis, 2014).

| Soil parameter | Status | Critical level | Soil parameter | Status | Critical level |
|---|---|---|---|---|---|
| | Very low | - | | Very Low | <0.5 |
| | Low | <25 | | Low | 0.5-1 |
| Fe (ppm) | Optimum | 25-300 | Cu (ppm) | Optimum | 0.9-20 |
| | High | 300-400 | | High | 20-30 |
| | Very high | >400 | | Very high | >30 |
| | Very low | < 1 | | Very low | <0.5 |
| | Low | 1-1.5 | | Low | 0.5-0.8 |
| Zn (ppm) | Optimum | 1.5-10 | B(ppm) | Optimum | 0.8-2 |
| | High | 10-20 | | High | 2-4 |
| | Very high | >20 | | Very high | >4 |
| | Very low | <60 | | | |
| | Low | 60-100 | | Low | < 0.05 |
| Mn(ppm) | Optimum | 100-300 | Mo(ppm) | Optimum | 0.05-0.1 |
| | High | 300-500 | | High | >0.1 |
| | Very high | >500 | | | |

Source: EthioSIS team analysis (2014).

color, crop residue management, rate of last year's fertilizer application and fertilizer type were recorded for each plot.

**Sample preparation and soil laboratory analysis**

The collected soil samples were air-dried, grounded and passed through 2 mm and 0.5 mm diameter sieves for analysis using wet chemistry conventional laboratory methods and spectral methods, respectively. Selected soil physical and chemical properties were analyzed at the National Soil Testing Center (NSTC) in Addis Ababa and the Mehlich-III extractable elements at Yara International Soil Laboratory in London.

Soil pH in $H_2O$ (1:2) was determined by using digital pH meter with glass electrode (Miller and Kissel, 2010). Extractable micronutrients (Fe, Zn, Mn, Cu, B and Mo) of the soils were extracted by Mehlich-III multi-nutrient extraction method (Mehlich, 1984) and were measured with their respective wave length range by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). Particle size distribution was analyzed by laser diffraction in wet mode of analysis with HORIBA-Partica (LA-950V2). The analysis of soil samples was run in a wet mode using 1% sodium hexametaphosphate (Calgon) solution as dispersing agent (Agrawal et al., 1991).

Organic carbon was predicted from MIR spectra of soil samples. Soil organic matter (SOM) was estimated by multiplying the soil organic carbon by 1.72 (Nelson and Sommers, 1996; Baldock and Skjemstad, 1999). The different values for the various soil micronutrients were rated using the EthioSIS critical levels (EthioSIS, 2014) as shown in Table 1 that it prepared based on extensive literature review.

**Data analysis**

The micronutrients values of the soil samples were interpreted from analytical data according to EthioSIS critical levels (Table 1). Simple linear correlation analysis was carried out between micronutrients and some governing factors of micronutrients status by using SAS software 9.1 version. The descriptive statistics such as mean, mode, median, SD and range were carried out separately for Fe, Zn, Mn, Cu, B and Mo. Finally, the micronutrient status was reflected in the maps. Based on Karltun et al. (2013), determination of critical level for Mn depends on soil pH. The empirical formula used to calculate critical levels of manganese activity index (MnAI) was

MnAI=101.7+3.75*Mn -15.2*pH

Where MnAI is manganese activity index; pH is pH of soil in water and Mn is the Mehlich-III extracted manganese (Karltun et al., 2013).

**Soil fertility mapping**

Ordinary Kriging was used to predict unknown soil nutrients' values for non surveyed areas based on the nearby sampled data. Point data of selective soil attributes were interpolated. For every soil property, sample distribution and variability was evaluated using the experimental variogram. Among the Exponential, Spherical and Gaussian models, the best fitted model to these experimental variograms was chosen using the lowest sum square errors (SSE). Using the lowest SSE, the best model was chosen and predictions over the study area were carried out for each soil micronutrients by using Arc GIS software version 10 with output pixel size of 100 x 100 m. After kriging was carried out for selective soil parameters, very low, low, optimum, high and very high nutrients status classes were defined from the map based on the EthioSIS critical rating values (EthioSIS, 2014).

The spatial dependence between samples was also determined considering the relationship between the nugget effect ($C_0$) and sill ($C_0 + C_1$) expressed in percentage: 0-25% high, 25-75% medium and 75-100% low spatial dependence between samples, as proposed by Cambardella et al. (1994).

**Table 2.** Descriptive and geo statistics of some soil micronutrients in the study districts.

| Woreda | Descriptive statistics | *Fe* | *Zn* | *B* | *Mn* | *Cu* | Mo |
|---|---|---|---|---|---|---|---|
| | | (mg kg$^{-1}$) | | | | | |
| Kedida Gamela (N=156) | Mean | 129.29b | 8.39$^{b}$ | 0.45 | 150.62$^{a}$ | 1.09b | 10.85$^{a}$ |
| | Std Dev | 38.14 | 5.76 | 0.32 | 54.37 | 0.32 | 2.95 |
| | Median | 128.79 | 6.50 | 0.32 | 144.0 | 1.0 | 10.99 |
| | Minimum | 50.04 | 1.3 | 0.02 | 41.0 | 0.6 | 3.06 |
| | Maximum | 209.72 | 28 | 1.83 | 330.0 | 2.9 | 18.71 |
| | CV (%) | 29.50 | 68.61 | 71.11 | 36.1 | 28.85 | 36.79 |
| Kecha Bira (N=147) | Mean | 146.73a | 14.45 a | 0.38 | 145.12ab | 1.19a | 6.84$^{c}$ |
| | Std Dev | 32.25 | 9.05 | 0.23 | 51.76 | 0.39 | 1.81 |
| | Median | 147.70 | 12.80 | 0.34 | 142.0 | 1.1 | 6.69 |
| | Minimum | 60.08 | 0.90 | 0.04 | 26.0 | 0.5 | 2.21 |
| | Maximum | 240.52 | 47.8 | 1.44 | 295.0 | 1.44 | 10.56 |
| | CV (%) | 22.02 | 62.63 | 59.59 | 35.67 | 32.84 | 26.48 |
| Damboya (N=155) | Mean | 147.11a | 4.78$^{c}$ | 0.36 | 133.7b | 1.31a | 9.62$^{b}$ |
| | Std Dev | 41.73 | 4.63 | 0.28 | 223.0 | 0.3 | 27.35 |
| | Median | 146.5 | 3.6 | 0.29 | 121.0 | 1.3 | 9.62 |
| | Minimum | 46.84 | 1.0 | 0.06 | 36.0 | 0.8 | 2.99 |
| | Maximum | 412.23 | 39 | 2.03 | 304.0 | 3.4 | 17.65 |
| | CV (%) | 28.37 | 96.86 | 77.78 | 39.02 | 22.90 | 28.24 |
| Total (N=458) | Mean | 141.06 | 9.11 | 0.40 | 143.24 | 1.2 | 9.15 |
| | Std Dev | 38.53 | 7.77 | 0.28 | 53.26 | 0.35 | 3.05 |
| | Median | 142.56 | 6.25 | 0.32 | 135 | 1.2 | 9.06 |
| | Minimum | 46.84 | 0.9 | 0.02 | 26.0 | 0.5 | 2.21 |
| | Maximum | 412.23 | 47.72 | 2.03 | 330.0 | 3.4 | 18.71 |
| | CV (%) | 27.31 | 85.22 | 70.63 | 37.18 | 28.98 | 33.37 |
| | Model | Exponential | Exponential | Exponential | Spherical | Spherical | Spherical |
| | Range (m) | 10125 | 6765.05 | 6291 | 1089 | 6883.71 | 19,144.70 |
| | $C_0/C_0+C_1$ | 0.46 | 0.53 | 0.70 | 0.60 | 0.56 | 0.45 |
| | Spatial dependence | moderate | moderate | Moderate | moderate | Moderate | Moderate |
| | $F_{value}$ | 9.14$^{***}$ | 40.66$^{***}$ | 2.32$^{ns}$ | 3.82$^{*}$ | 9.53$^{**}$ | 96.74*** |

Numbers in the brackets refer to sample size; *, **, *** significant at p<0.05 p < 0.01, 0.001, respectively.

## RESULTS AND DISCUSSION

The descriptive and geo statistics of soil micronutrients of soil samples collected from Kadida Gamela, Kachabria and Damboya districts are shown in Table 2.

### Iron status

The Melich III extractable Fe varied from 50.04 to 209.72 mg kg$^{-1}$ with a mean value of 129.29 mg kg$^{-1}$, 60.08 to 240.52 mg kg$^{-1}$ with a mean value of 146.3 mg kg$^{-1}$ and 46.84 to 233.32mg kg-$^{1}$ with a mean value of 145.39 mg kg$^{-1}$ in agricultural soils of Kedida Gamela, Kecha Bira and Damboya districts, respectively as shown in Table 2. Statistically significant difference (p<0.0001) was observed in mean values among the districts and moderate variability (CV= 27.31%) existed among Fe data. The highest and lowest mean values 147.11 and 129.29 mg kg$^{-1}$, respectively of extractable Fe content were recorded in Damboya and Kedida Gamela distrcts.

According to the critical level adopted by EthioSIS (2014), almost all of agricultural soil of Kedida Gamela, Kecha Bira and Damboya districts, were found to be optimum in Fe status.

Also, Figure 2 shows the Fe status that was predicted from measured sites by using co-Kringing. Exponential model was found to be the best fit for Fe data and range value for Fe was 10125 m. The nugget to sill ratio was 0.46 confirming the existence of moderate spatial

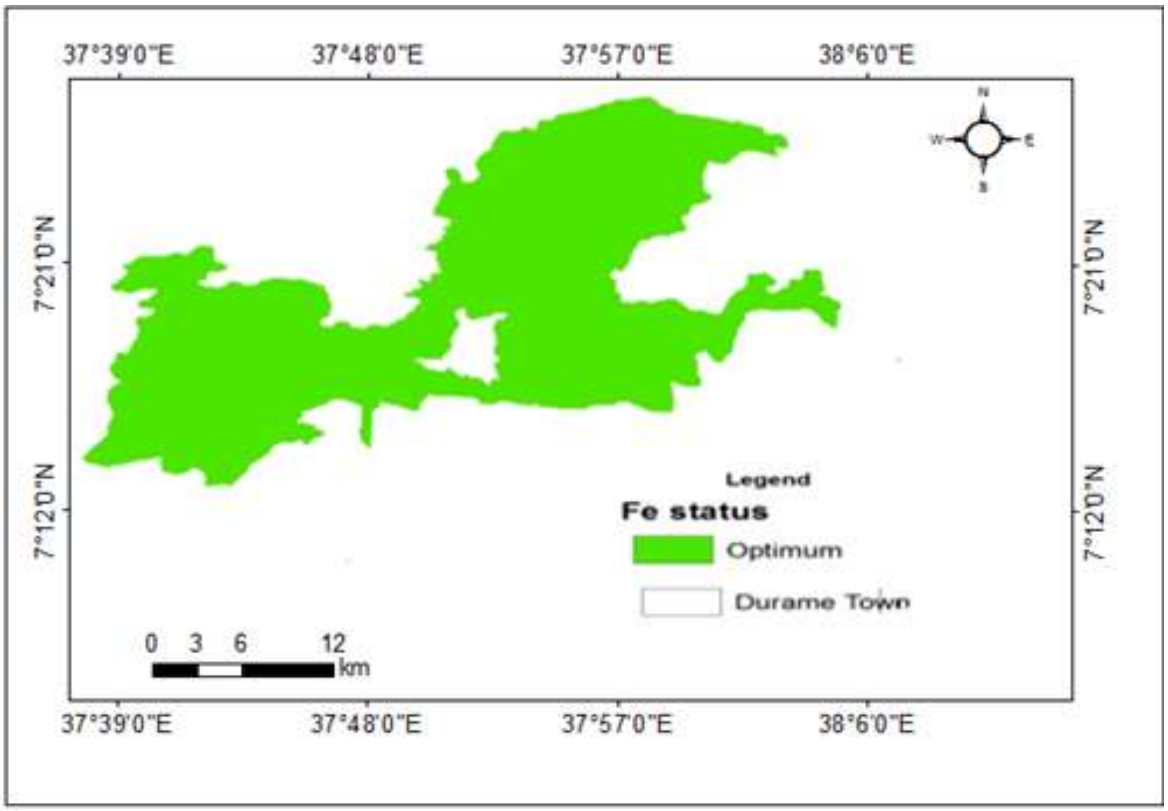


**Figure 2.** Soil extractable Fe map of the study area.

dependence between Fe dataset. It was observed that in terms of area coverage, all of the agricultural lands were found to be optimum in extractable Fe status. This finding is in agreement with the results of Haque et al. (2000), Abayneh (2005), Eyob et al. (2015) and Hilette et al. (2015) who reported that Fe was adequate in the soil samples collected from different regions of the country. On the other hand, Teklu et al. (2007) reported that 20 % of Vitric Andisols collected from Rift Valley of Ethiopia were deficient in Fe. Similarly, Yifru Abera and Mesifn Kebede (2013) reported Fe deficiency in 96% of soil samples collected from central highlands of Ethiopia. Also, EthioSIS fertility mapping project reported that Fe was deficiency in some Tigray agricultural soils (EthioSIS, 2014).

The existence of adequate Fe content in the soils may be due to the parent material that contains minerals like Feldspar, Magnetite, Hematite and Limonite which together constitute the bulk of trap rock in these soils (Vijaya Kumar et al., 2013). Also, soil reaction (pH) of the study area may contribute to the high amount of extractable Fe since the pH of the majority of soils in the study area is less than 7 that can enhance the solubility of Fe. Diatta (2014) and Diatta et al. (2014) reported that soil reaction (pH) is of prime importance in controlling towards the availability of micronutrients, since it affects directly their solubility as well as activity in the soil environment.

## Zinc status

As shown in Table 2, extractable Zn widely ranged from 0.3 to 28 (mean = 8.39), 0.9 to 47.8 (mean= 14.45), and 1 to 39.2 (mean = 4.78) mg $kg^{-1}$, for agricultural soils of Kedida Gamela, Kecha Bira and Damboya Woredas, respectively. The mean separation showed that means were significantly different among districts ($P<0.001$).The highest mean value for extractable Zn content (14.45 $mgkg^{-1}$) was recorded in Kecha Brira district whereas the lowest mean value (8.39 $mgkg^{-1}$) was recorded in Kedida Gamela districts.

From the frequency distribution (Fig. 3A), and referring to the critical level adopted by EthioSIS, (2014) (Table 1), the majority (66.03% of Kedida Gamela, 37.41% of Kehca Bira and 84.52% of Dambya woredas') agricultural soils were optimum in Zn status. The remaining 26.8% of Kedida Gamela, 30.61% of Kech Bira and 6.45% of Damiboya districts agricultural soils were found to be high in extractable Zn status. Also, 26.28% of Kedida Gamela, 30.61% of Kecha Bira and 1.94% of Dmboya woredas agricultural soils were found to be very high in Zn status.

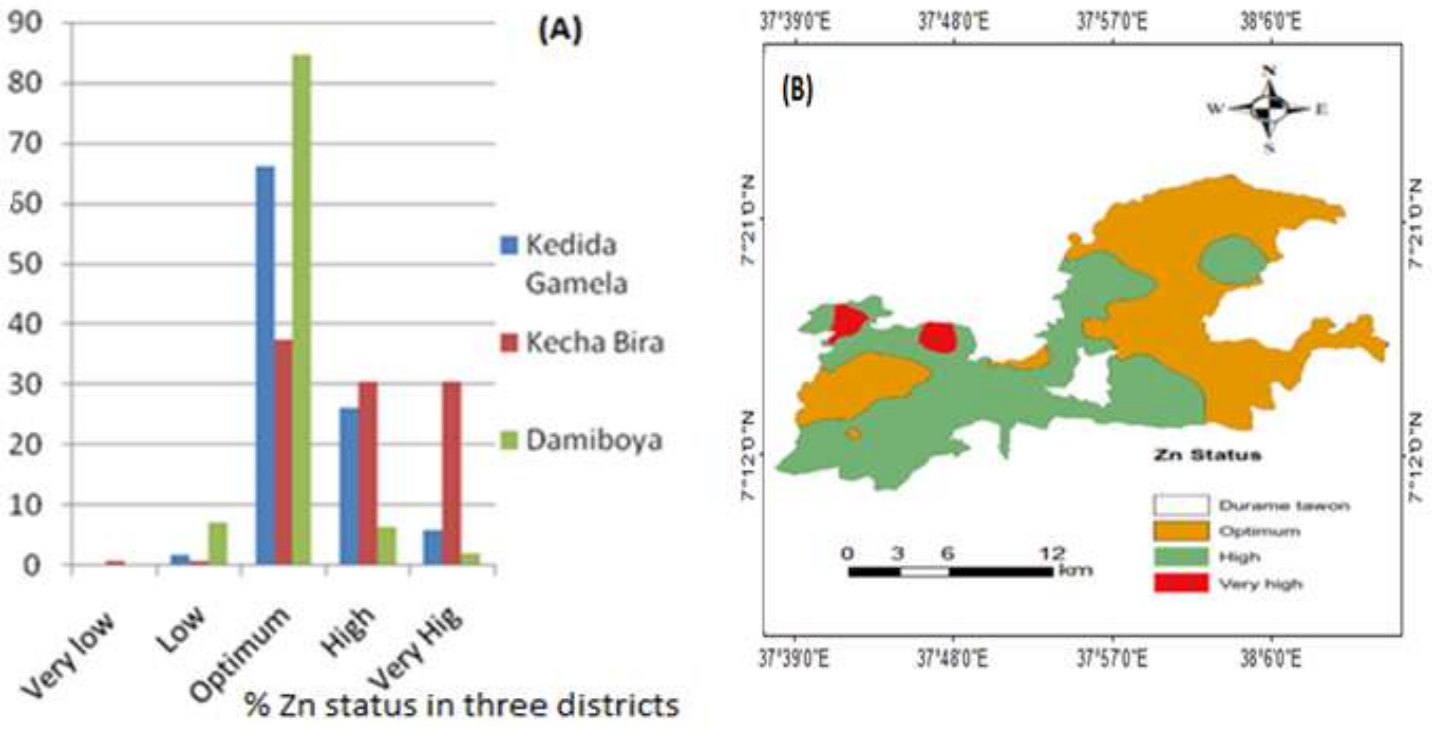


**Figure 3.** (A) Status of Zn (B) Soil extractable Zn map of the study areas.

Moreover, little proportion (1.92% of Kedida Gamela, 1.36% of Kacha Bira and 7.1% of Damboya woredas') agricultural soils were found to be below optimum level in extractable Zn status. Also, out of 147 samples of KechaBira district, only two soil samples (one each) were very low and low in Zn status.

Zinc status of unmeasured fields was predicted by interpolation using ordinary krining method. Exponential method was found to be the best fit on semivariogram of Zn. The range and sill to nugget ratio were found to be 6765.05 m and 0.53, respectively indicating moderate spatial dependent between Zn data. Figure 3B shows that in terms of area coverage, 61896.49, 65217.81 and 1690.569 ha were found to be optimum, high and very high in Zn status. The result of this study shows that Zn was in sufficient range in soils of all the study districts. This may be due to the soil conditions such as low pH and parent materials of soil that are high in Zn content. Certain soil conditions reduce the availability of Zn, notably high pH (Jones, and Eck, 1973). Thus, a high incidence of Zn deficiency often occurs on calcareous or limed soils. The present study soils were neither limed nor calcareous and the pH values in the majority of the soils were not too high to precipitate available Zn.

## Manganese status

As shown Table 2, the Melich III extractable Mn ranged from 41 to 330 mg $kg^{-1}$ for Kadida Gamela, 26 to 295 mg $kg^{-1}$ for Keca Bira and 16 to 300 mg $kg^{-1}$ for Damboya districts agricultural soils. Its concentration has reached at level of toxicity to affect most of the crop species (Jones, 2003). Statistically significant difference ($P<0.05$) was observed in mean values among the districts and moderate variability (CV= 28.98%) existed among Mn data. The highest and lowest mean values 150.62 and 133.7 mg $kg^{-1}$, respectively of extractable Mn content were recorded in Kedida Gamela and Damboya districts. The range and sill to nugget ratio were found 1089 m and 060, respectively indicating moderate spatial dependent between Mn data.

The calculated manganese activity indexes (MnAI) of soil samples ranged from 141-1252 for Kadida Gamela, 123-1130 for Keca Bira and 159-1142 for Damboya districts. According to Karltun et al. *(*2013), critical level for MnAI is 25. When the MnAI status of the soils of the study area was compared with the critical level, it was 5 to 50 times more than the critical level. This indicated that Mn toxicity is one of the factors that contribute to the low crop production and productivity in the study woredas. The result of this study is in line with the finding of Eyob Tilahun et al.(2015) and Wondwosen Tena and Sheleme Beyene (2011) who reported that amount of extractable Mn are generally high in the tropical soils and Mn toxicity is even more common than deficiency. Liming can be used to reduce Mn extractability and availability of Mn.

The existence of higher amount of Mn in soils of the

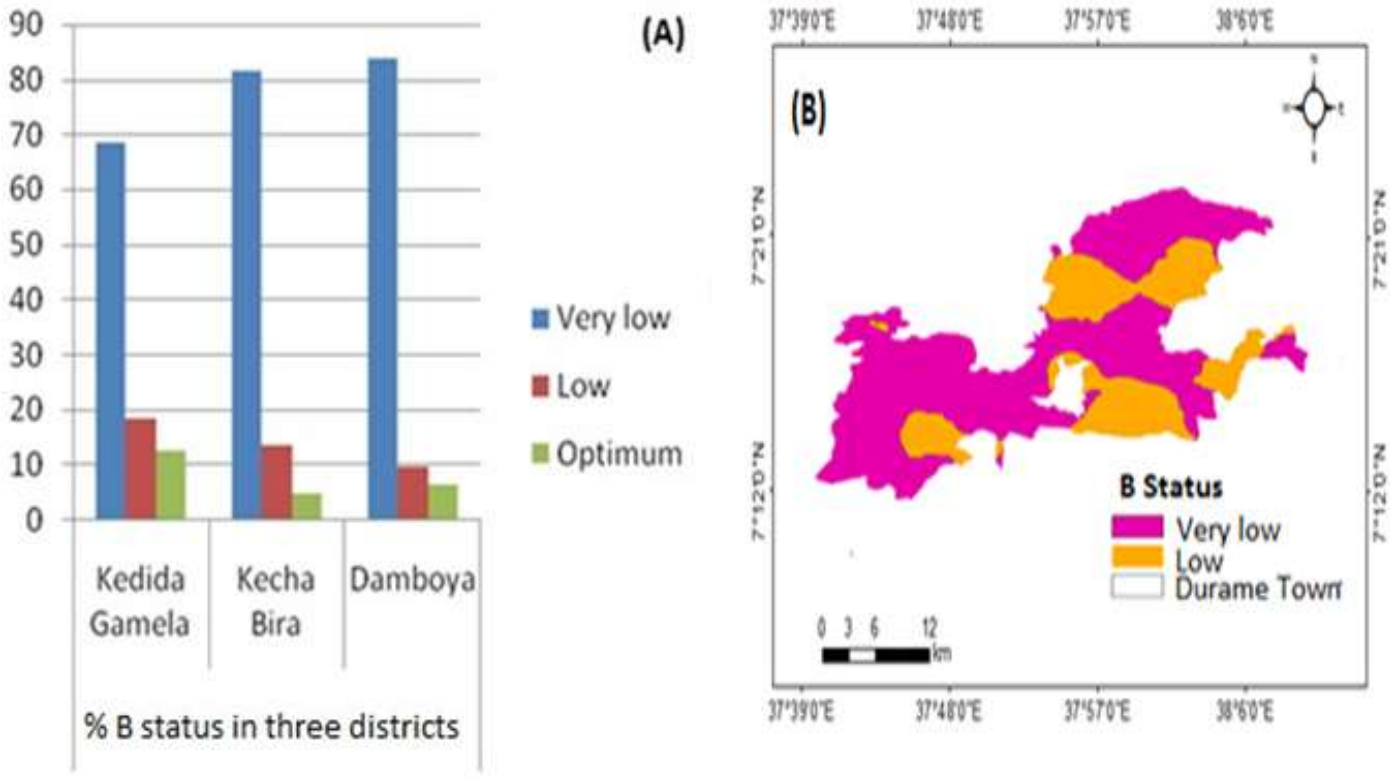


**Figure 4.** (A) Frequency distribution of B status of the three woredas (B) B status map.

study area may be due the weathering of primary Mn-containing ferromagnesium minerals that form secondary minerals such as pyrolusite ($MnO_2$). According to Jones (2003), Mn is also found in soils as Mn oxides, in part adsorbed at the surfaces of clay minerals.

## Boron status

Melich III extractable B varied from 0.02 to 1.83 mg $kg^{-1}$, 0.4 to 1.44 mg $kg^{-1}$ and 0.06 to 2.00 mg $kg^{-}$ in agricultural soils of Kedida Gamela, kecha Bira and Damboya districts, respectively (Table 2).The mean B value was found to be 0.45 ppm for Kedida Gamela, and 0.38 ppm for both Kacha bira and Damboya districts and means are not significantly different ($p>0.05$). The larger CV (>50%) indicates that there was higher variability between B data, mainly due to variation in landscape positions, management practices, land use types, soil type and inherent properties like soil texture and pH. Also, random sampling of large number of samples from vast areas could result in moderate to high soil variability.

As shown in the frequency distribution (Figure 4A) , the majority of soils (68.59% of Kedida Gammela, 81.63% of Kacha bira and 83.87% of Damboya districts were found to be very low in Melich III extractable B status. The other 18.59, 13.61 and 9.68% of Kedida Gamela, Kecha Bira and Damboya districts' agricultural soils were respectively, found to be low in B status. A few proportion (12.82% of Kedida Gamela, 4.76% of Kecha Bira and 6.45% of Damboya districts') agricultural soils were found to be optimum. The exponential model was found to be the best fit for B data. The range value 7290.08 m and nugget to sill ratio 0.71 indicates that the spatial structure for B data is moderate.

Area coverage in different status of B was calculated after prediction of all areas by using co-kiring method by spherical model as shown in Figure 4B. Accordingly, in terms of area coverage, 90880.78 ha (70.56%), 37864.09 ha (29.39%), 60.0 ha (0.001%) of the agricultural soils in the study districts were very low, low and optimum, respectively. This revealed that nearly all agricultural soils of the study areas were below critical level in B status and it is one of the crop yield limiting nutrient in the study areas. The result of this study is in line with that of Wondwosen Tena and Sheleme Beyene (2011) and Eyob Tilahun et al. (2015) who reported that B was deficient in some soils of western and southern Ethiopia.

The possible reasons for B deficiency in the study area may be due to loss of B through leaching in acidic soil, low B absorbing capacity of soils, low OM, continuous cultivation of soils, low B containing parent materials, lower application rate of manure and use of non B containing fertilizer (Oyinlola and Chude, 2010; Chesworth,

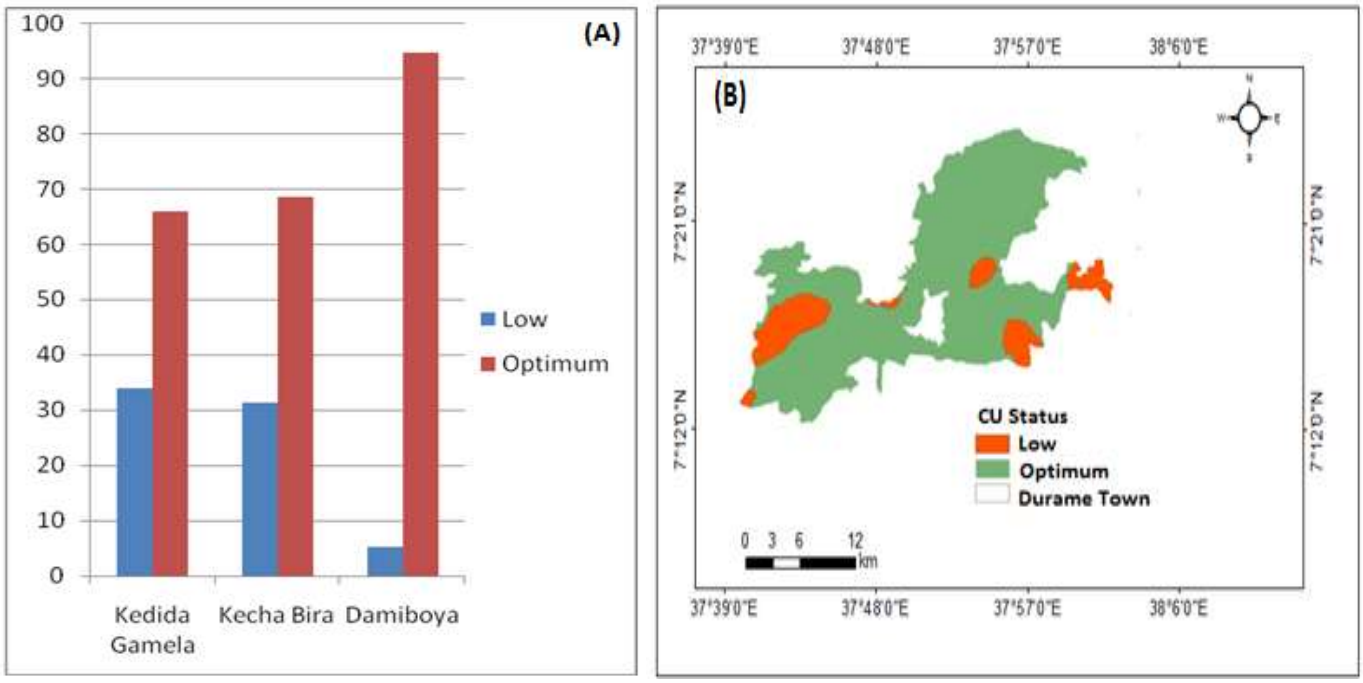


**Figure 5.** (A) Frequency distribution of Cu status of the three districts (B) Cu status map.

2007). Generally, this finding revealed that B is deficient and it may be one of yield limiting elements in all soils of the three woredas since its deficiency affects the growing points of roots, shoots and young leaves and retard the uptake of calcium (Tandon, 1997). Therefore, it is strongly recommended that B should be included in blended or compound fertilizer to boost the crop yield in the study area.

## Copper status

As shown in Table 2, extractable Cu ranged from 0.6 to 2.9, 0.5 to 1.44 and 0.8 to 3.4 mg $kg^{-1}$ in agricultural soils of Kedida Gamela, Kecha Bira and Damiboya districts, respectively. The mean Cu values were found to be 1.09, 1.119 and 1.31 mg $kg^{-1}$ for Kedida Gamela, Kecha Bira and Damboya districts, respectively.

As shown in the frequency distribution (Figure 5A) and referring the Cu critical levels, adopted by EthioSIS (2014), the majority of agricultural soils of the study areas (66.03% of Kedida Gamela, 68.71% of Kecha Bira and 94.84% of Damboya districts) were found to be optimum in Melich III extractable Cu status. The remaining 33.70% of Kedida Gamela, 31.29% of Kecha Bira and 5.16% of Damboya woredas were found in low category of Cu status.

Exponential model provided the best fit for the semivariogram of Cu. The range for Cu on semivariogram was 6884.71 m (Table 2). The nugget to sill ratio 0.56 indicates that the spatial dependence of extractable Cu was moderate. The prediction map (Figure 5B) indicates that in terms of area coverage, 11 and 89% of soils were found to be low and optimum, respectively in Cu status as per of critical level adopted by EthioSIS (2014).

The finding of this study revealed that 11% of agricultural soils in the study woradas were deficient in Cu. This may be due to low soil OM, intensive cropping systems and non use of Cu containing fertilizer which could result in high Cu mining from the soils. Soils derived from coarse-grained sediments (sands and sandstones) as well as acid igneous rocks are usually low in Cu. According to Harmsen.and Vlek (1985), factors affecting the soils ability to provide Cu to plants include pH, humus content and proportion of sand to clay (Harmsen and Vlek, 1985). The findings of this study revealed that 11% of agricultural soils should be supplemented Cu containing fertilizers in order to improve crop yields since most plants are sensitive to Cu deficiency. Cereals (oats, wheat, barley, maize) and vegetables are particularly sensitive (Murphy and Walsh, 1972).

## Molybdenum status

The Melich III extractable Mo varied from 3.06 to 18.71 mg $kg^{-1}$(mean 10.85 mg $kg^{-1}$), 2.21 to 10.52 mg $kg^{-1}$ (mean 6.84 mg $kg^{-1}$) and 2.99 to 17.56 mg $kg^{-1}$ (mean 6.92 mg $kg^{-1}$) in agricultural soil of Kedida Gamela, Kecha Bira and Damboya districts, respectively as shown in

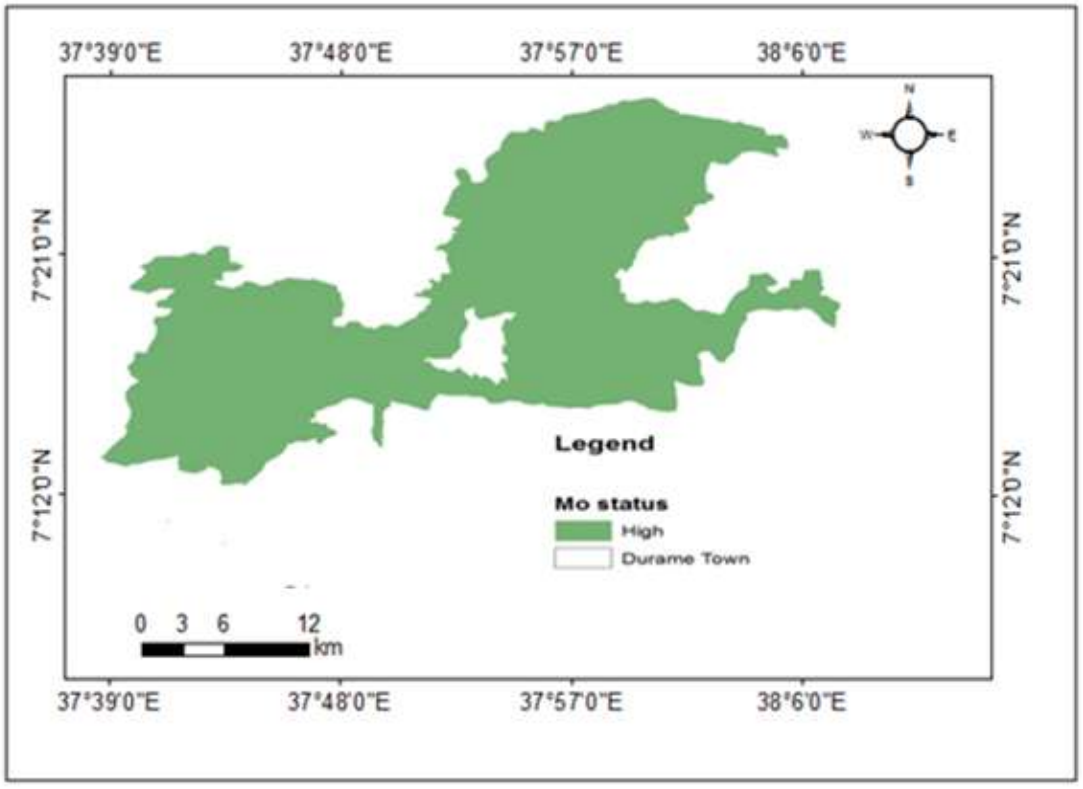


**Figure 6.** Soil extractable Mo map of the study area

Table 2. Statistically significantdifference ($p<0.0001$) was observed in mean values among the woredas and moderate variability (CV= 33.71%) existed among Mo data. This indicates that soil characteristics in the woredas differ greatly and such manifestation of differences in extractable Mo content in the soils might be ascribed to the larger variation in soil characteristics such as pH, calcium carbonate and soil texture, which greatly influence the amount of extractable Mo in soils, and its eventual availability to growing plants (Gupta and Dabas, 1994; Adhikari et al., 1997; Sharma et al., 2003).

According to the critical level adopted by EthioSIS (2014), almost all of agricultural soils of Kedida Gamela, Kecha Bira and Damboya districts were found to be high in Mo status. This might be linked with the stage of weathering of soils. Availability of Mo in young volcanic soils is generally high (Mengel and Kirkby, 2001). Figure 6 shows the Mo status predicted from measured site. Spherical model was found to be the best fit for Mo data and range value for Mo was 19,144.7 m. The nugget to sill ratio 0.45 indicates that the spatial dependence of extractable Cu was moderate.

Regardless of high status of extractable Mo in the soils of study area, for future application of balanced fertilizers together with high-yielding varieties would lead to Mo deficiency. For instance, in India introduction of high-yielding varieties and higher use of nitrogen, phosphorus and potassium, increased crop production many folds and led to Mo deficiency in 11% of Indian soils (Singh, 2001). Similarly, Gupta and Lipsett (1994) reported that applying S has been found to decrease uptake of Mo by plants. Therefore, in order to judge the status of Mo in the study area soils, further study is recommend after harvesting high crop yields in the near future since Mo is one of the important micronutrients which helps in biological N fixation in legumes and legumes are given special consideration in the study region.

## Relationship between available micronutrients and some soil properties

The micronutrient content of soils is influenced by several factors among which soil organic matter content, soil reaction and clay content are the major ones (Fisseha, 1992). Therefore, an attempt was made to examine the relationship between copper, zinc, boron and molybdenum and some soil properties (pH, organic matter and particle size) by simple correlation analysis (Table 3), to identify the soil factors involved in regulation of amounts of extractable Cu, Zn, B and Mo in soils. Significant and positive ($p<0.001$) relationship of extractable Fe, Zn, B and Cu with organic matter ($r$ = 0.21, 0.43, 0.12 and 0.13), respectively was observed

**Table 3.** Correlation between some soil properties and extractable micronutrients in soils of study areas.

| Extractable micronutrients | Soil properties | | | | |
|---|---|---|---|---|---|
| | pH | OM | Clay | Silt | Sand |
| Fe | -0.43** | 0.212** | -0.3 | 0.13* | -0.11* |
| Zn | -0.07 | 0.43** | -0.056 | 0.061 | 0.098* |
| Mn | -0.1* | 0.009 | -0.87 | 0.079 | 0.062 |
| B | 0.32** | 0.123** | -0.168** | 0.11* | 0.172** |
| Cu | 0.22** | 0.129** | -0.09* | 0.16** | -0.037 |
| Mo | 0.18** | -0.05 | -0.55*** | 0.62*** | 0.25*** |

(Table 3). The results were in close agreement with findings of Yadav (2011); Khalifa et al*.* (1996), Eyob Tilahun et al. (2015) and Kumar et al*.* (2013). The reason for this might be the ability of SOM to form natural chelates that can maintain micronutrients in an available form. Also, organic matter controls the affinity, attraction strength of micronutrients with most functional groups (Jean et al*.*, 2014).

The negative correlation of extractable Fe, Zn and Mn with soil pH was observed. This indicates that there is precipitation of extractable micronutrients into insoluble products when pH rises. The activity of Mn, Fe, and Zn decreases 100-fold for each unit increase in soil pH (Lindsay, 1978). Various correlation studies by Rajagopal et al. (1977) and Haldar and Mandal (1979) have confirmed that decline in extractable Zn associated with the rise in pH. Many researches revealed that soil pH is negatively correlated with Fe content (Wang et al*.,* 2009; Sharma et al., 2004; Najafi-Ghiri et al*.,* 2013). According to Katyal et al. (1982), Zn deficiency was generally observed in crops growing on alkaline soils. Ismunadji et al. (1982) reported that Fe chlorosis was severe in several crops growing on high pH calcareous upland soils of Indonesia. The availability of micronutrients Fe, Zn and Mn decreases as the soil pH increases due to the hydrolysis reactions (through the splitting of water molecules in their hydration shells) (Sinskey, 2009). Boron and Mo were positively and significantly related with pH, silt and sand but negatively correlated with clay. Chavan et al. (1980) also noticed an increase in water-soluble B with fineness of texture. While Le Mare (1970) stated that B deficiency occurs on light textured soils. Goldberg (1993) stated that sorption of B to Fe and Al oxides was pH dependent and was highest at pH 6 to 9 and bioavailability of B was highest between pH 5.5 to 7.5, decreasing below 8.5. According to Singh (1970), high B and Mo contents were noticed in saline alkaline soils.

## CONCLUSION AND RECOMMENDATION

This study showed that the Melich III extractable Fe, Zn and Mo status in most of agricultural soils of the study woredas was found to be sufficient. The calculated MnAI was greater than the critical value that indicates the Mn toxicity is common in the soils of study areas. Extractable B is below optimum level in most of soil samples analyzed and it might be one of the yield limiting nutrients in the study areas. Majority of soil samples analyzed were optimum in Cu status but about 11% of soil samples analyzed were low in Cu status.

Further, the contents of micronutrients (Fe, Zn, Mn, Cu and B) increased with increase in organic matter content this might be due to organic matter content may supply chelating agents. In order to boost crop yield, fertilizers that contain B for all soils of study the areas and Cu for 11% of the study area soils should be recommended. Moreover, the geo-referenced sampling sites can be revisited after a few years 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 .The study can be strengthened by further analysis of plant tissue samples taken from field grown crops in the study areas.

## Conflict of Interests

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Growth and physiology of peanut (*Arachis hypogaea* L.) irrigated with saline water and biofertilizer application times

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**The peanut crop has adaptive features to high salinity levels in the soil, due to its morphological and physiological characteristics. The objective of this study was to evaluate the use of biofertilizer in reducing the effects of irrigation water salinity on the vegetative and physiological behavior of the peanut crop, as well as the crop development under saline conditions. The experiment was conducted in a greenhouse, in an experimental design with randomized blocks, adopting a 6 × 3 factorial scheme, concerning the irrigation water salinity (CEa) in six levels (0.5, 1.5, 2.5, 3.5, 4.5 and 5.5 dS $m^{-1}$) and three doses of biofertilizer (applied at 15, 30 and 45 days after germination), with four replications, totaling 72 experimental units. The variables analyzed were: plant height; fresh and dry weight of shoots; stem diameter; length, fresh weight and dry weight of roots; number of branches; leaf area; photosynthetic radiation, chlorophyll a, b, and total chlorophyll. The use of biofertilizer had no influence on reducing the effects of irrigation water salinity in the development of the peanut crop. Salinity negatively affected all physiological and growth variables.**

**Key words:** Electric conductivity, salinity, organic input, *Arachis hypogaea*.

## INTRODUCTION

Peanut (*Arachis hypogaea* L.) originates from South America, belonging to the group of oil plants of the family Fabaceae. It is a plant that grows well in different types of weather and temperature, showing to be well adapted to hot and wet seasons. In the semiarid region, it is seen as a profitable alternative for small producers (Silva et al., 2011a).

To enable the exploitation of this crop in different

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ecosystems, it is necessary to know the behavior of physiological parameters when subjected to different types of stress (Erismann et al., 2006; Graciano et al., 2011).

It has great importance in the world grain market and according to the database of the United States Department of Agriculture (USDA, 2015); the world production of peanut in the 2014/2015 season was 6,654,000.00 tons, with a planted area of 23,654,000.00 ha, while Brazil contributed with 346,800.00 tons, harvested in an area of 121100 ha (Conab, 2016). Asian countries are the main producers, with outstanding leadership exercised by China, being an important product in the economy of these countries. According to the data of the Food and Agriculture Organization (FAO) of the United Nations, peanut production is led by China, India and the United States, and these countries hold about 80% of the world production (FAO, 2011). In Brazil, production is mainly concentrated in the Southeast, Midwest and Northeast (Conab, 2016).

One of the major environmental factors limiting agricultural productivity due to its effects on plant growth and development is the soil salinity, which can be ionic and/or osmotic in nature, since the electrical conductivity of saline soils is equal to or greater than 4.0 dS $m^{-1}$, which corresponds to an approximate concentration of 4 nM NaCl and an osmotic pressure of 0.2 MPa (Munns and Tester, 2008).

In the case of the northeast, the salinization of soils is attributed to the fact that the potential evapotranspiration usually has lower values than the rainfall (Silva and Amaral, 2007). This feature, coupled with inadequate management of water and soil, has affected the productivity of the crops grown in the region. The increase in the salt content of the irrigation or soil water decreases the osmotic potential of the solution, reducing the availability of water and nutrients to plants (Sousa et al., 2010).

According to Graciano et al. (2011), in salt stress conditions, the peanut crop develops physiological mechanisms to ensure its growth, a fact considered an adaptive strategy.

Hence, the search for management strategies that facilitate the exploration of areas irrigated with saline water is a challenge that is being gradually overcome, highlighting the use of substances that reduce the intensity of the damaging effects of salt, allowing the use of saline water during seedling and plant growth (Sousa et al., 2008), the use of different water sources in different plant development stages (Neves et al., 2009) and the use of organic conditioners based on bovine biofertilizer (Cavalcante et al., 2010).

In the case of use of biofertilizers, research has been developed as an alternative used to mitigate the deleterious effects of salinity on soil and plants, and this use of bovine biofertilizer is shown to mitigate the effects of salt stress on the initial growth of some crops (Medeiros et al., 2011; Cavalcante et al., 2011).

Given the earlier, the aim of this study was to evaluate application times of biofertilizer in reducing the effects of irrigation water salinity on the vegetative and physiological behavior of the peanut crop, as well as the crop development under saline conditions.

## MATERIALS AND METHODS

The work was carried out in a screened greenhouse in the Agriculture Sector of the Center of Social/Human and Agricultural Sciences of the Federal University of Paraíba - Campus III, Bananeiras-PB (CCHSA/UFPB) in the period from January to May, 2014. The UFPB - Campus III is located in the meso region of the Agreste Paraibano and in the micro region of Brejo Paraibano, 130 km away from the capital João Pessoa and 70 km away from Campina Grande - PB. With an altitude of 526 m, Bananeiras-PB has wet and cold weather, with an average temperature of 28°C in the summer and 15°C in the winter (IBGE, 2006).

According to the Köppen classification, the climate is of the As' type - tropical rainy, with dry summer, irregular annual rainfall distribution (1174.7 mm), maximum annual temperature of 27°C and minimum of 18.8°C, and getting an annual average of 22°C (AESA, 2011).

The experimental design was a randomized block, adopting a 6 × 3 factorial scheme, referring to six levels of salinity of the irrigation water (CEa) (0.5, 1.5, 2.5, 3.5, 4.5, and 5.5 dS $m^{-1}$) and three biofertilizer application times (at 15, 30 and 45 days after germination), with four replications, totaling 72 experimental units.

The experimental units were comprised of pots with volume capacity of 12 $dm^3$, being packaged, in these containers, 800 g of crushed stone 1 (base), and subsequently the substrate composed of humus (70%), sand (20%) and manure (10%). The substrate was analyzed for its characteristics according to the methodology described by Embrapa (1999) (Table 1). The substrate acidity correction was preceded by applying 35 g of dolomitic limestone and 32 g of phosphorus ($P_2O_5$) was indicated as base fertilization (Alvarez et al., 1999).

The planting was done with peanut cultivar BR1, placing five seeds per pot; after 15 days, thinning was held, leaving only one plant per pot.

Irrigation was carried out in the late afternoon, with irrigation turn of 3 days, from the sowing, up to 22 days, being reduced to 2 days in order to replace the crop evapotranspiration, estimated for each development stage of the plant from the reference evapotranspiration (ETo) and the crop coefficient (Kc). ETo values were obtained in the AESA platform (Executive Agency for Water Management in the State of Paraíba), where the water depth was adjusted for each irrigation. The water preparation, with its respective salt levels (CEa), was performed weekly by diluting saline water ($C_4S_1$) with non-saline water (C1S1), stored in 50 $dm^3$ containers (Choi et al., 2005).

The bovine biofertilizer was prepared by anaerobic fermentation, by adding 100 $dm^3$ of fresh manure and 100 $dm^3$ of water (CEa ≤ 0.5 dS $m^{-1}$) in a container with capacity of 240 $dm^3$. The system was kept sealed for 30 days until reaching a pH close to 7 (Santos and Akiba, 1996). To release the methane gas produced by the fermentation, one end of a thin tube was connected to the top of the biodigester and the other was submerged in a container with water to prevent the entry of air.

In the periods corresponding to the biofertilizer application on the substrate (15, 30 and 45 days after germination - DAG), a further dilution was performed, in the ratio of 1:1 (biofertilizer and water with CEa ≤ 0.5 dS $m^{-1}$), being applied to the surface of the substrate, a quantity corresponding to the volumes of each

**Table 1.** Characterization chemical and fertility of the substrate used.

| *pH | P | $K^+$ | $Na^+$ | $H^+Al^{+3}$ | $Al^{+3}$ | $Ca^+$ | $Mg^{+2}$ | SB | CTC | V | M.O. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $H_2O$ | mg $dm^{-3}$ | | | | | $cmol_c$ $dm^{-3}$ | | | | % | g $kg^{-1}$ |
| 5.8 | 63.65 | 0.76 | 0.21 | 0.71 | 0.00 | 5.76 | 3.26 | 9.99 | 10.7 | 93.36 | 36.13 |

*Water pH; SB = Sum bases (Ca2 + + Mg2 + + K + + Na +); CEC = cation exchange capacity [SB + (H + + Al3 +); V = soil bases exchangeable saturation (SB / CTC) 100; MO = Organic matter.

**Table 2.** Summary of analysis of variance related to plant height (AL), stem diameter (DM), number of branches (NR), root length (CR), fresh matter of shoot (MFPA), dry mass of (MSPA), fresh root mass (MFR), root dry mass (MSR), interaction of photosynthetic radiation (IRF), chlorophyll a (Cla), chlorophyll b (Clb) and chlorophyll (Clt) of peanut plants (Arachis hypogaea L.), depending on the salinity of the irrigation water and different times of application Biofertilizer.

| FV | GL | Mean square | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AL | NR | DM | CR | MFPA | MSPA | MFR | MSR | IRF | Cl*a* | Cl*b* | Cl*t* |
| S | 5 | 2487.82** | 99.71** | 23.12** | 292.88** | 55609.60** | 3299.51** | 90.70** | 7.46** | 0.99** | 1386.08** | 144.83** | 2425.20** |
| B | 2 | 326.26** | $1.02^{ns}$ | $5.83^{ns}$ | $155.01^{ns}$ | $9536.62^{ns}$ | $672.75^{ns}$ | $16.71^{ns}$ | $1.02^{ns}$ | $0.15^{ns}$ | $376.32^{ns}$ | $38.03^{ns}$ | $644.92^{ns}$ |
| S × B | 10 | 195.12** | 25.45** | $2.50^{ns}$ | 13.57* | $3625.70^{ns}$ | $178.08^{ns}$ | $6.22^{ns}$ | $0.44^{ns}$ | 0.03* | $63.05^{ns}$ | $10.88^{ns}$ | $118.46^{ns}$ |
| R | 54 | 54.38 | 4.58 | 2.47 | 49.19 | 5273.95 | 326.53 | 17.06 | 1.13 | 0.12 | 150.16 | 15.66 | 255.62 |
| Total | 71 | - | - | - | - | - | - | - | - | - | - | - | - |
| CV (%) | - | 13.91 | 15.96 | 0.71 | 18.85 | 18.44 | 18.09 | 17.23 | 7.55 | 11.92 | 13.51 | 12.66 | 13.25 |

Salinity - ECw (S) Biofertilizer (B) residue (R); CV = Coefficient of variation; ns = not significant by F test; *,**Significant respectively at 5 and 1% probability.

treatment. Growth variables (plant height, stem diameter, root length, fresh and dry weight of shoots, fresh and dry weight of roots, number of branches, leaf area index) and physiological variables (photosynthetic radiation, chlorophyll a, b and total chlorophyll) were evaluated.

The results were submitted to analysis of variance by F test at 0.05 probability and in cases of significance, polynomial regression analysis was performed (Banzatto and Kronka, 2006), using the statistical software ASSISTAT, version 7.7 beta (Silva and Azevedo, 2002).

## RESULTS AND DISCUSSION

Through the data in Table 2, it is observed that the variables plant height, stem diameter, number of branches, root length, fresh weight of shoots, dry weight of shoots, fresh weight of roots, dry weight of roots, leaf area index, photosynthetic radiation, chlorophyll a, chlorophyll b and total chlorophyll had significant effects with the different salinities in the irrigation water. Regarding the biofertilizer application times, only the variable plant height obtained significance, while the others showed no significant effect. The different salinity × biofertilizer ratios showed significant interaction effect only for the variables plant height, number of branches, root length and photosynthetic radiation.

From the regression analyses concerning plant height, depending on the electrical conductivity of water (CEa) under different biofertilizer application times (Figure 1), it was found that the increase in CEa reduced plant height in the three applications of the organic input, with higher decrease from CEa = 4.5 dS $m^{-1}$. The application at 45 DAG showed major development of plants with CEa values between 0.5 and 1.5 dS $m^{-1}$. Researchers state that the decrease in plant height can occur due to decreased osmotic potential of the soil solution due to the increase in salinity levels (Graciano et al., 2011). To confirm this statement, Sousa et al. (2012), irrigating the peanut crop with saline water, reported trends similar to this study for this variable.

Regarding the number of branches, plants showed a decrease with increasing CEa (Figure 2). The growth inhibition is caused, mostly, by the toxic effects of the salts absorbed by plants, by the low osmotic adjustment capacity of the crop

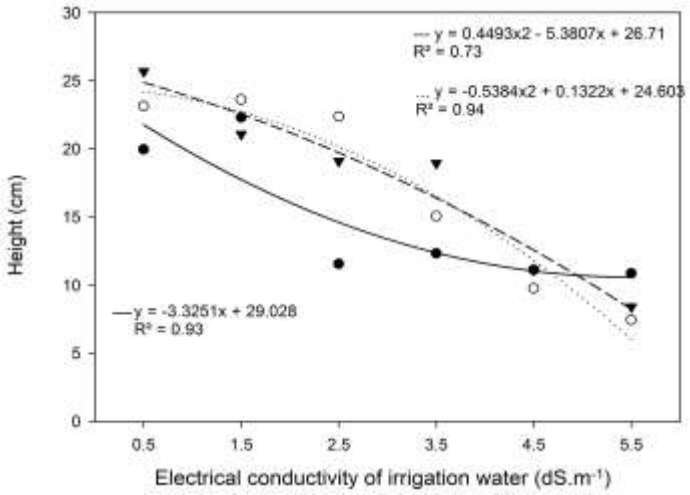


**Figure 1.** Height of peanut plants irrigated with saline water and bio-fertilizer application times to 15 (-), 30 (--) and 45 (··) days after germination.

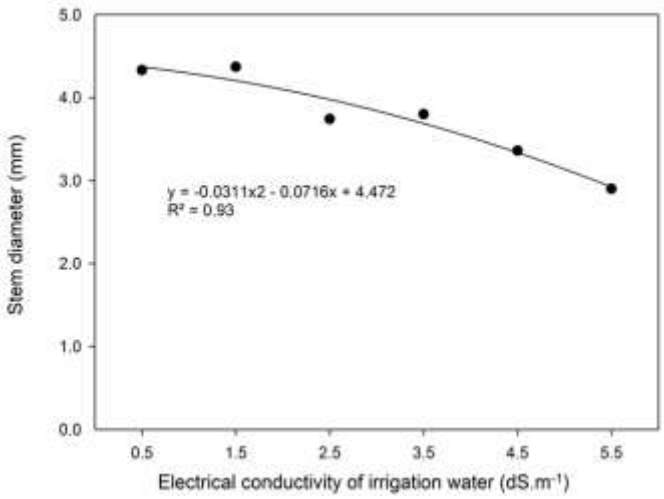


**Figure 3.** Stem diameter of peanut plants irrigated with saline water.

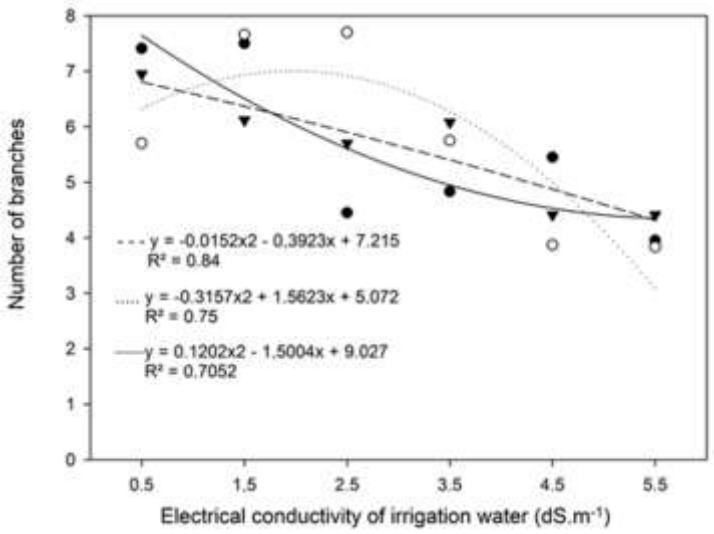


**Figure 2.** Number of branches of peanut plants irrigated with saline water and bio-fertilizer application times to 15 (-), 30 (--) and 45 (··) days after germination.

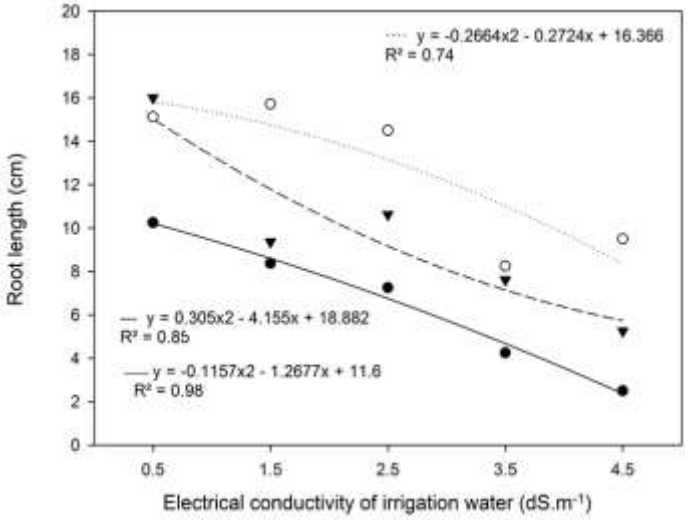


**Figure 4.** Root length of peanut plants irrigated with saline water and bio-fertilizer application times to 15 (-), 30 (--) and 45 (··) days after germination.

and by the reduction of the total water potential caused by increased salt concentration (Lacerda et al., 2006; Silva et al., 2011b).

With respect to stem diameter (Figure 3), increasing CEa reduced the development thereof in the presence of biofertilizer. Correia et al. (2005) found similar behavior of this variable for the peanut crop, testing increasing levels of salinity in the irrigation water. Moreover, Campos et al. (2009), in castor beans, Medeiros et al. (2011), in cherry tomato, and Nascimento et al. (2011), in pepper, found superiority of this variable in plants irrigated with increasing levels of salt in the irrigation water in the presence of biofertilizer.

The root length of the peanut was inhibited for the three biofertilizer applications (Figure 4), however, the application at 45 DAG provided favorable conditions for further development of roots. To the extent that the CEa increased, the root length of plants decreased. Work involving biofertilizers and saline water showed no significant effect for this variable, as reported by Campos et al. (2009), in castor beans, and Medeiros et al. (2011), in cherry tomato.

According to the data of Figure 4, it was observed that the fresh weight of shoots (Figure 5A) showed a decrease.

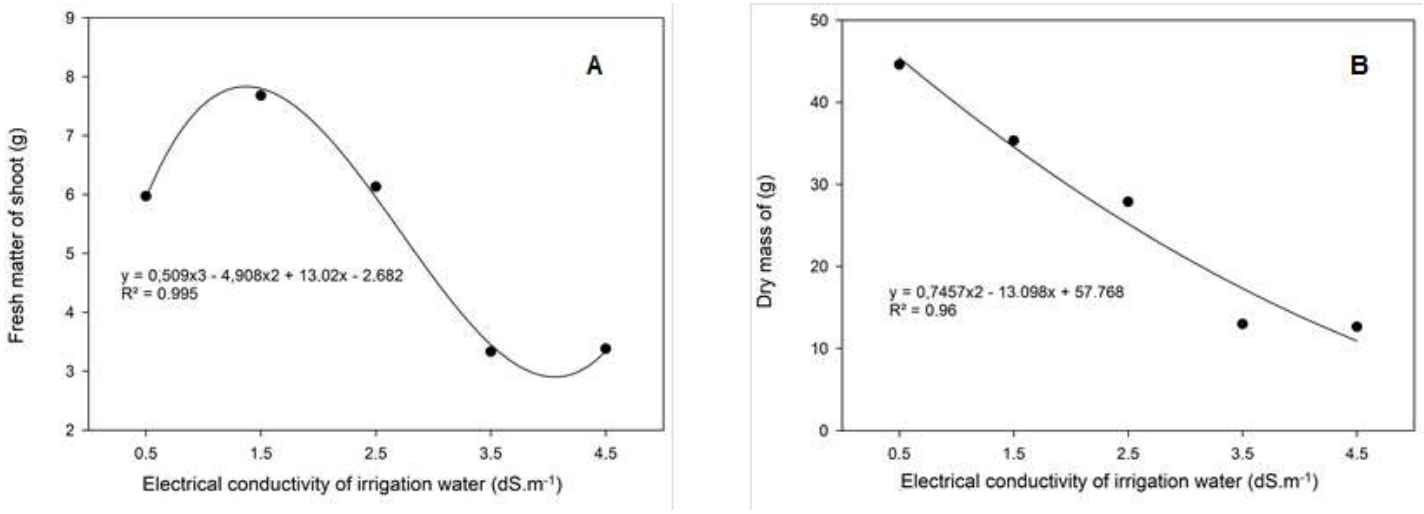


**Figure 5.** Fresh matter of shoot (A) and dry mass of (B) of peanut plants irrigated with saline water.

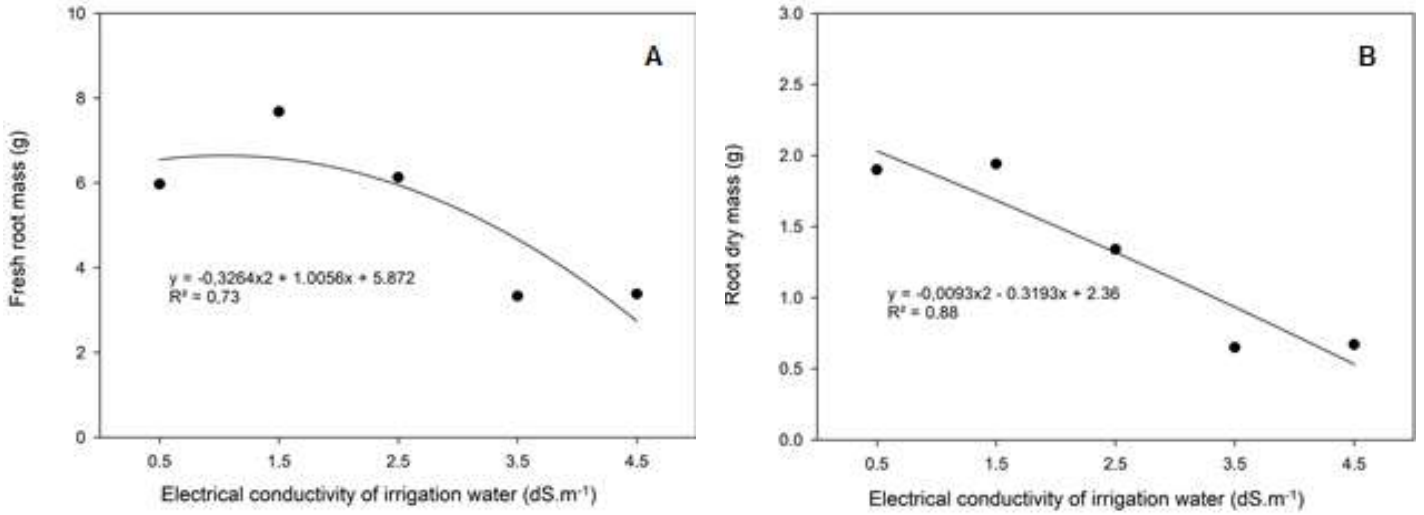


**Figure 6.** Fresh root mass (A) end root dry mass (B) of peanut plants irrigated with saline water.

This occurred due to the use of saline water for irrigation, as this water influences the development of the plant. This negative effect of salinity results in lower efficiency of plants in photosynthetic processes and in the transport of organic solutes in plant tissues, and as a consequence, in the growth and development of their tissues (Figueiredo, 2012). The dry weight of shoots (DWS), as a function of the irrigation CEa (Figure 5B), was influenced by the conductivity of the water. It showed a decrease at all salinity levels. Similar results were shown by Graciano et al. (2011) for the peanut crop. Corroborating this information, Morais et al. (2011), in sunflower plants, and Silva et al. (2011b) in cowpea, also reported reduction in DWS when increasing concentrations of salts were applied in the irrigation water.

The fresh weight of roots was similar for water doses of 0.5 and 1.5. In starting this conductivity, a greater decrease was presented (Figure 6A). This reduction is justified by the fact that excessive salinity reduces the growth of all parts of the plants, as it causes an increase in energy expenditure to absorb water from the soil and perform biochemical adjustments necessary for their survival under stress conditions (Larcher, 2006). Regarding the dry weight of roots, it decreased with increasing electrical conductivity of the water used for irrigation (Figure 6B). These results are contrary to those reported by Silva et al. (2011a), which showed an increase in the accumulation of dry root mass with increasing biofertilizer doses in the cotton crop. This

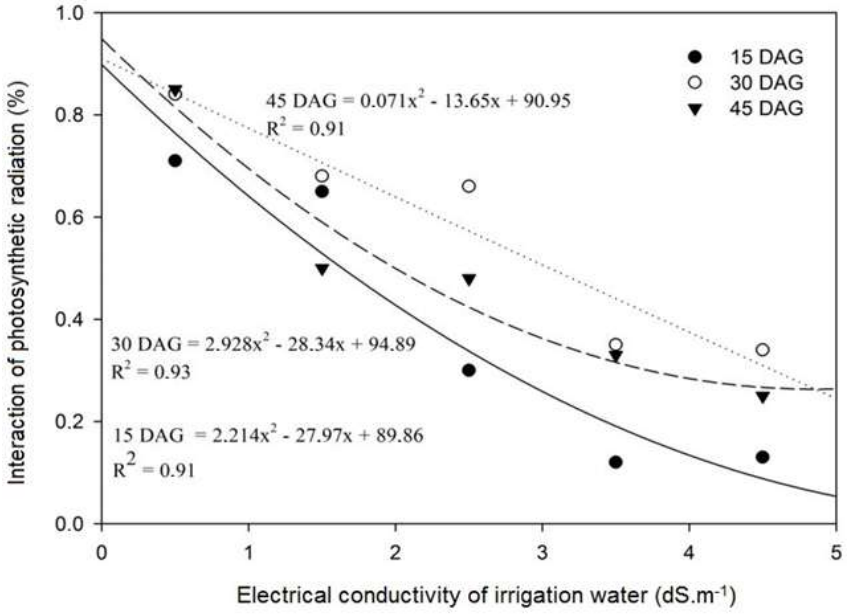


**Figure 7.** Interception of photosynthetic radiation of peanut plants irrigated with saline water and bio-fertilizer application times to 15 (-), 30 (--) and 45 (··) days after germination.

evidence of reduction in the dry weight of roots has been a classic behavior seen in other studies when plants are subjected to salt stress (Blanco et al., 2008; Medeiros et al., 2011; Maciel et al., 2012).

The biofertilizer application did not influence the analyzed variables; notwithstanding, there wasinteraction between the water doses and the biofertilizer. Possibly, in view of the decrease in the size of plants and leaves, the plant reduced the transpiration surface and the exposed area in order to intercept photosynthetically active radiation (Figure 7), probably due to the increase of the ABA (abcisic acid) concentration in the xylem. This induces stomatal closure in the leaf and reduced leaf expansion, as these are extremely sensitive to lack of water, being affected, even before there is interference in the leaf water potential, by the phytohormone balance (Kramer and Boyer, 1995). Water absorption is also sensitively affected by abiotic factors such as the salinity of the medium, because as the water transport is mediated by aquaporins, these channels are selective to water and independent of energy expenditure for their operation (selection via molecular size) (Steudle and Henzler, 1995), which will reduce their photosynthetic potential and hence their productivity (Ávila et al., 2007). The peanut has C3 photosynthetic metabolism and features maximum net photosynthetic rate at 30°C. The maximum dry matter production rate, or crop yield, is 19.6 $gm^{-2}$ day (Embrapa, 2009).

Increased CEa provided a reduction in the index of chlorophyll a, b and total chlorophyll (Figure 8). This may be related to physiological conditions of stress, such as

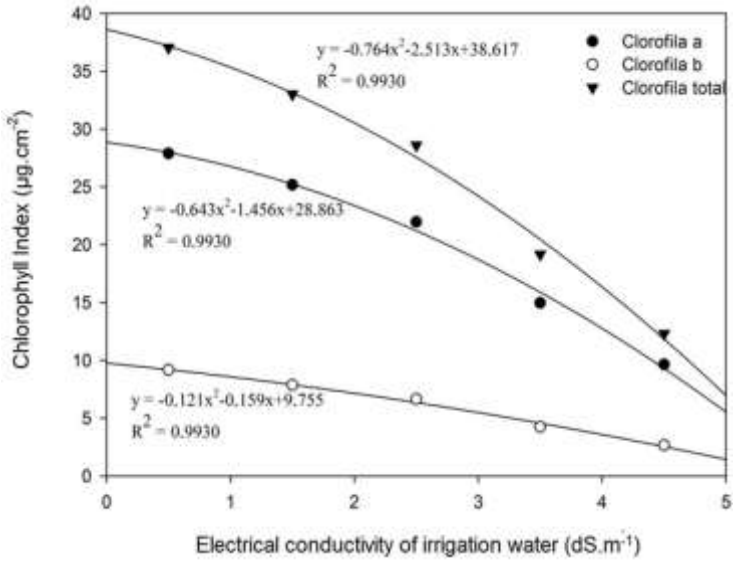


**Figure 8.** Chlorophyll a, chlorophyll b and chlorophyll total of peanut plants irrigated with saline water.

salinity, lack of water and nutrient deficiency; it is possible, in these cases, that the ferredoxin transfers its $e^-$ to the molecular $O_2$, forming $H_2O_2$, which must be reduced by catalase, generating more ATP without NADPH, which is the pseudo-cyclic electron transport. This process also has the function of producing additional ATP and consuming molecular $O_2$, in photoinhibition

conditions. $O_2$ has two antagonistic effects on photosynthesis, one protective, by the use of NADPH and ATP when produced in excess under photoinhibition, by photorespiration and by Mehler reaction; and one destructive, by the action of active oxygen species such as $H_2O_2$. These active oxygen species destabilize the membranes, for example, the thylakoids (Vácha, 1995), which may cause a decrease in the content of photosynthetic pigments, as CEa reduces the chlorophyll content in plants sensitive to salinity (Jamil et al., 2007; Silva et al., 2008; Graziano et al., 2011). The reduction in the chlorophyll content as a function of the salinity effect was also observed in sugarcane (Willadino et al., 2011). High concentrations of NaCl increase chlorophyll degradation via chlorophyllase and decrease its synthesis, by virtue of the competition for nitrogen with other compounds, such as proline (Ibarra and Maiti, 1995). The chlorophyll degradation may cause a considerable reduction in the photosynthetic rate and, as a result, decreased productivity (Santos, 2005).

## Conclusions

The use of biofertilizer had no influence on reducing the effects of irrigation water salinity in the development of the peanut crop. The application of biofertilizer at 45 days after germination promoted more vegetative growth and increased photosynthetic activity in the peanut crop. Salinity adversely affected all physiological and growth variables, which showed a decrease with increasing electrical conductivity of the irrigation water (dS $m^{-1}$).

## Conflicts of Interests

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Irrigation water management during the ripening of tomato aiming fruit quality

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**Despite the shortage of water resources be evident worldwide, turning prosperous lands into unproductive and affecting the existence of humidity, there is a great fraction of producers who still do not implement the proper management of the irrigation water in their properties. The poor management of irrigation is still a reality for most of the producers located in Brazil *Middle Cerrado*, which continue irrigating the tomato crop using empirical methods, resulting in economic, environmental and social damage. Therefore, the objective of this study was to evaluate the influence of different irrigation depths and different times of suspension in irrigation during the ripening stage in tomato in productivity and quality of fruit for processing. The experiment was conducted under a Center pivot irrigation system and a split plot experimental design. The study showed that the higher irrigation depth (100% of ETc) resulted in higher yield (104 t ha$^{-1}$) with lower soluble solids. The irrigation suspension at the beginning of fruit maturation increased soluble solids accumulation (4.2°Brix), providing higher quality and production of fruits to tomato industry.**

**Key words:** Productivity, *Solanum lycopersicom*, L., water deficit.

## INTRODUCTION

The tomato (*Solanum lycopersicum* L.) has a great economic importance because its dual purpose. This vegetable can be freshly consumed or used for industrial processing, being one of the main consumed vegetables in the world. In 2012, Brazil occupied the ninth position in the world ranking of producers of tomato, producing about 3.8 million tons in an area of 55.5 thousands hectares, approximately (Agrianual, 2015).

The crop cycle of tomato in the Middle Cerrado of Brazil lasts on average, 115 days. According to Marouelli et al. (2012), the consumption of water during the development cycle of tomato is between 300 and 650 mm. Analyzing the irrigation in tomato for processing, Silva and Marouelli (1999) found that productivity increased by 25% when the

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**Table 1**. Results of physical and hydraulic analysis of soil of the experimental area.

| Parameter | Unit | Depths (cm) | |
|---|---|---|---|
| | | 0-20 | 20-40 |
| FC[1] | $m^3\ m^{-3}$ | 0.198 | 0.180 |
| PWP[2] | $m^3\ m^{-3}$ | 0.098 | 0.081 |
| Clay | dag $kg^{-1}$ | 15.50 | 16.56 |
| Silt | dag $kg^{-1}$ | 5.25 | 2.30 |
| Fine sand | dag $kg^{-1}$ | 3.25 | 3.22 |
| Coarse sand | dag $kg^{-1}$ | 1.00 | 1.00 |
| Gravel | dag $kg^{-1}$ | 75.00 | 72.00 |
| Pebbly | dag $kg^{-1}$ | 0.00 | 5.00 |

[1]Field capacity and [2]Permanent wilting point.

irrigation management was carried out by monitoring soil moisture with neutron probe and, or, based on the crop evapotranspiration.

The anticipation in irrigation withholding has been reported by some authors (Marouelli and Silva, 1993; May, 1998) as alternatives to minimize the occurrence of bacterial and fungal diseases and reduce the rotting and disuniformity of fruits during ripening. This advance also contributes to the increasing of total soluble solids levels and enhance the efficiency of water use during the cultivation of tomato, mainly in sprinkler irrigation system.

The fruiting stage in tomato is considered the most sensitive stage to the deficit of water in soil. In this stage, an inadequate irrigation management can compromise productivity and quality of fruits (Marouelli et al., 1991; Prietro, 1997).

The hydric deficit during the maturation favors the increase of the total soluble solids content of fruits in tomato. In order to improve the quality of fruits, the management of irrigation should occasion a gradual reduction in the volume of the water applied from the ripening onset until the complete suspension of irrigation before harvest (Cahn et al., 2002).

It is important that the optimal time of suspension in the irrigation and the time of irrigation with deficit at the maturation of fruits be caried out in a local scale, considering the specific conditions of each region (Marouelli and Silva, 2009). This because the irrigation strategy in the maturation phase is dependent on various factors such as soil water holding capacity; if the cultivated genotypes are cultivar or hybrids; the effective depth of the root system; the irrigation system as well as the atmospheric evaporative demand (Marouelli and Silva, 2000; Lopez et al., 2001).

The reduced availability (López-Mata et al., 2010; Li et al., 2015) and global concern about water resources make necessary the adoption of strategies for the reduction of water use without reducing crop yields (Navarro et al., 2015). The water use efficiency (WUE) index can be used to plan and take decisions on irrigation (Karatas et al., 2009).

Considering all of these aspects, this study was caried out aiming to evaluate the influence of different irrigation depths and times of suspension of irrigation during the ripening stage of tomato, in productivity and quality of fruit for processing.

## MATERIALS AND METHODS

### Experimental field and crop management

The experiment was conducted in the municipality of Cristalina / GO, at an altitude of 922 m, latitude 16° 20 'S and longitude 47° 32' W, in 2009. The soil is classified as a red latosol, clayey-gravel texture (gravel above 50% in weight). In the Unified Soil Classification System (USCS), the soil texture is classified as GW (coarse gravel> 50%). It were caried out physical-hydric analyzes of the soil and the results are shown in the Table 1.

The tomato seedlings of the hybrid UG 8169 were purchased from reputable nurseries located in the region of the experiment and transplanted on 03/09/2009, with the harvest being held on 07/19/2009. The seedlings transplant was performed using the double rows system, with 1.2 and 0.6 m between rows and 0.3 m between plants, with a total population of 37,037 plants per hectare. The cultural management such as pest and disease control was performed weekly by applying the application of insecticides and fungicides, respectively.

### System and irrigation management

Irrigation was carried out using a center pivot system, having a Christiansen Uniformity Coefficient of 90% (Moazed et al., 2010). The irrigation depth was calculated using a water balance, wherein the water inlet was by irrigation and the water output by the crop evapotranspiration (ETc). The following equations proposed by Allen et al. (1998) were used to estimate the evapotranspiration.

$$ETc = ETo \times Kc \quad (1)$$

$$Kc = (Kcb \times Ks) + Ke \quad (2)$$

Where:
ETo – reference evapotranspiration, mm $day^{-1}$;
Kc – crop coefficient, dimensionless;
Kcb – crop basal coefficient, dimensionless;
Ks – stress coefficient, dimensionless; and
Ke – soil evaporation coefficient, dimensionless.

The mean values of Ks and Ke during the crop cycle are shown in Figure 1.

The crop cycle was divided into phenological stages based on the growth period, also known as vegetative period, related to the shading area where each stage assumed different basal crop coefficients (Kcb). This was necessary because the characteristics of the crop stages and edaphoclimatic conditions affect the values of Kcb in tomato (Allen and Pereira, 2009).

The first stage started after seedlings transplantation and extended until the point in which about 5% of the area was covered by the crop, which corresponded to a period of 10 days. The second stage, lasting 43 days, was completed when the crop covered about 100% of the area. The third stage of the crop began when 100% of the area wascovered and lasted until the beginning of fruit ripening, lasting 45 days. The last phase, with 20 days, started from the ripening of fruits and finished with the fruits

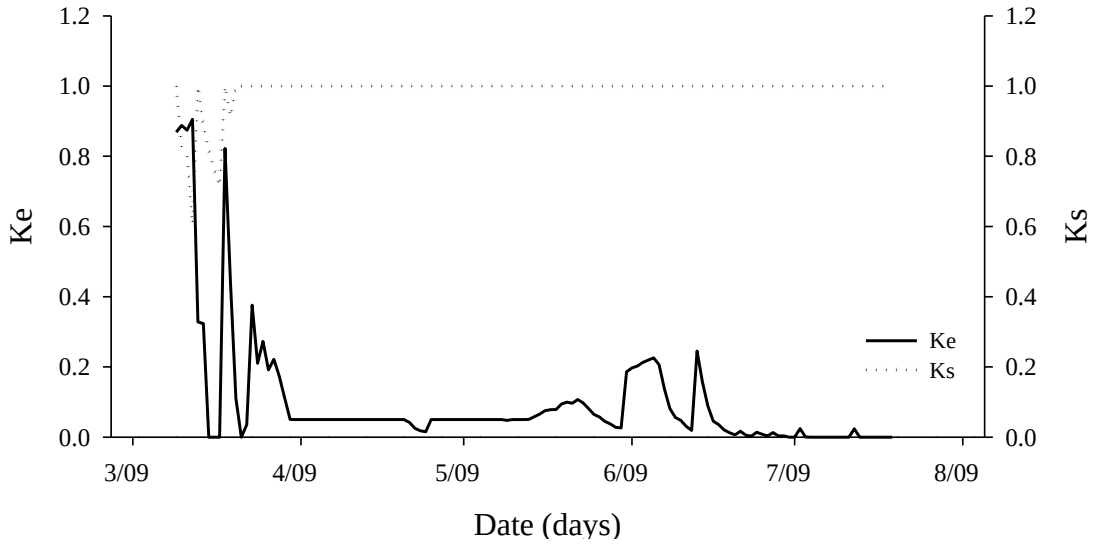


**Figure 1.** Stress coefficient (Ks) and soil evaporation coefficient (Ke) during the crop cycle.

**Table 2.** Precipitation and irrigation recorded during the crop cycle for each treatment.

| Treatments (% ETc) | Precipitation (mm) | Irrigation (mm) | Precipitation + Irrigation (mm) |
|---|---|---|---|
| 25 | 320 | 81 | 401 |
| 50 | 320 | 162 | 483 |
| 75 | 320 | 243 | 563 |
| 100 | 320 | 324 | 644 |
| S1 | 320 | 221 | 541 |
| S2 | 320 | 241 | 561 |
| S3 | 320 | 253 | 573 |

harvesting.

The ETc was determined based on the initial, intermediate and final Kcb values, with the respective values of 0.15, 1.15 and 0.70 (Allen et al., 2006).

The irrigation frequency and the total water quantity (irrigation + precipitation) varied between treatments (Table 2).

### Climate

As shown in Figure 2, the average temperature and precipitation during the experimental period were 20.9°C and 519.4 mm, respectively. The temperature during the cultivation remained in the recommended range for tomato (Ohnishi et al, 2010; Tarchoun et al., 2012.).

The average values of solar radiation and air relative humidity during the experiment were 17.7 MJ $m^{-2}$ and 70%, respectively and can be observed in Figure 3.

### Analyzed variables

It were evaluated the following agronomic characteristics: yield of commercial fruits, percentage of rotten fruits, pulp yield, total soluble solids content (TSSC), average weight of commercial fruits and number of fruits per plant.

For the evaluation of fruits, it were evaluated eight central plants of each replication, totalizing a useful area of 1.08 $m^2$. The fruits were divided into commercial and rotten fruits, followed by the percentages of each class in relation to the total yield of fruits. The pulp yield was estimated based on the yield of commercial fruits and $^0$Brix of each treatment, according to the equation bellow.

$$Y_{pulp} = \frac{(Y_{fruit} \times 0.95) \times °Brix}{28} \quad (3)$$

Where:
$Y_{pul}$ – pulp yield, t $ha^{-1}$; and
$Y_{fruit}$ – Fruits yield, t $ha^{-1}$.

The TSSC of ripe fruits was determined using a digital refractometer for measures from 0 to 45 °Brix. This appliance is recommended for the determination of sugar in concentrated fruit juices and canned products using sugar infusion. For this evaluation, a sample was collected from 100 fruits per replication, which was triturated using an industrial blender, followed by the °Brix measurement.

### Experimental design and statistical analysis

The experiment was conducted in a split plot design, having the in

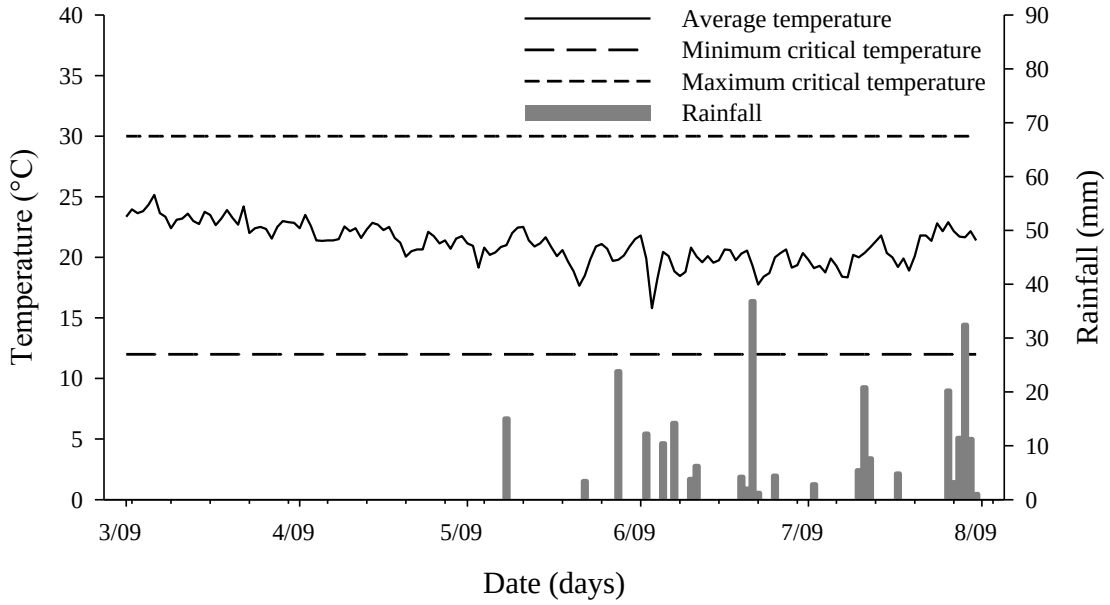


**Figure 2.** Critical values of maximum and minimum temperatues; average values of temperature and precipitation for the croping period of tomato in the municipality of Cristalina, Goiás, Brazil.

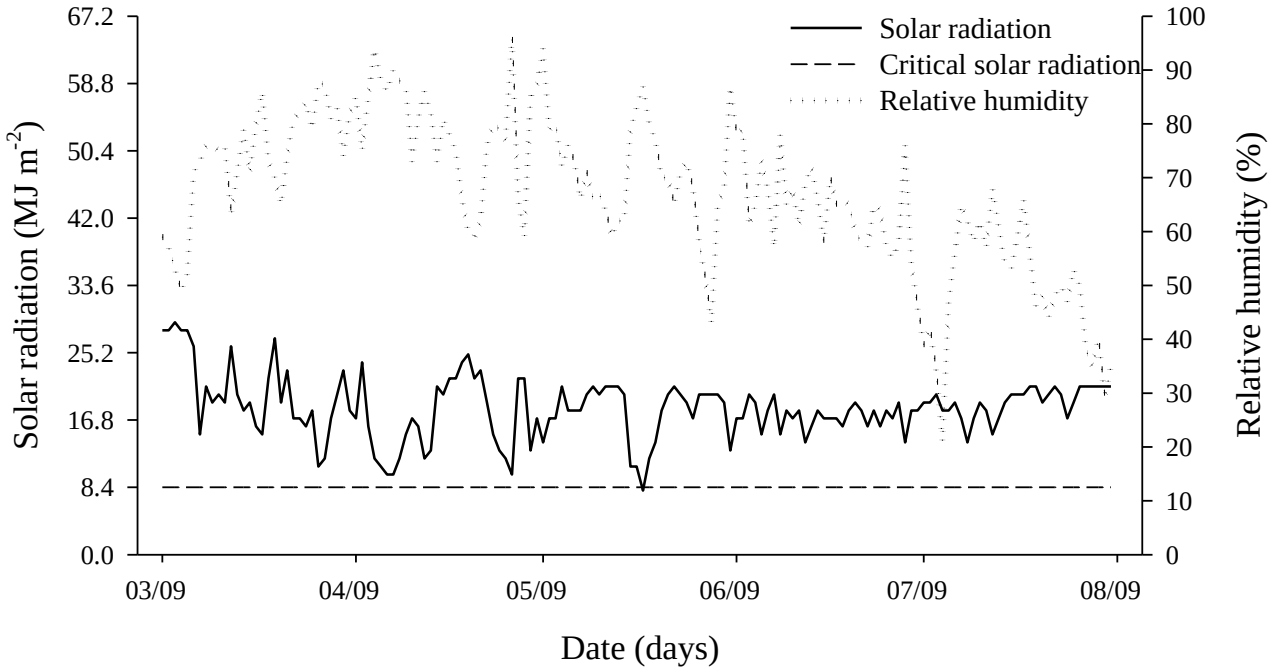


**Figure 3.** Solar radiation, critical solar radiation and relative humidity for the croping period of tomato in the municipality of Cristalina, Goiás, Brazil.

the plots the irrigation depths with 25, 50, 75 and 100% of the crop ETc and in the subplots the time of suspension of irrigation, when 10 (S2) 20 (S1) and 95% (S3) of plants had at least one ripe fruit. The experiment was conducted in a randomized complete block design with three replications.

Each experimental plot consisted of a double row of plants, 3.0 m long, totaling 20 plants. The experimental data was analyzed by variance analysis and regression. For the qualitative factor (Irrigation suspension), the means were compared using the Tukey test at 5 and 10% of probability. For the quantitative factor (irrigation depths), the models were chosen based on the significance of the regression coefficients using the "t" test at 1 and 5% of probability.

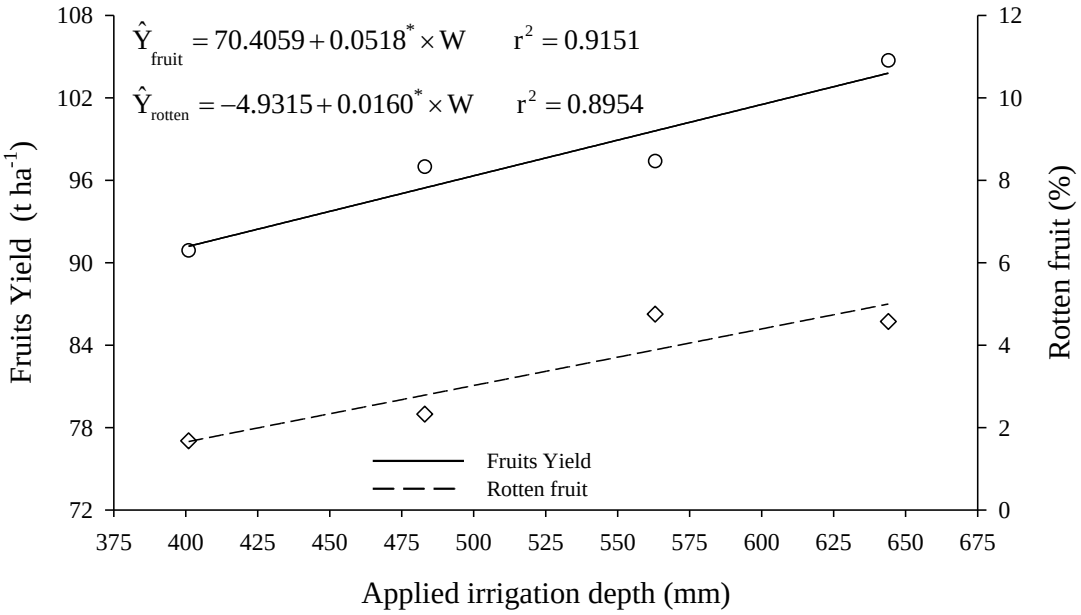


**Figure 4.** Fruit yield and percentage of rotten fruits as the result of the applied irrigation depths. *Significant level of 5% probability by Student "t" test.

## RESULTS AND DISCUSSION

As shown in Figure 4, it is observed that the yield of ripe fruit linearly increased upon the increasing of the irrigation depth. The highest productivity (104 t $ha^{-1}$) was obtained by applying the irrigation depth corresponding to 100% of the ETc (650 mm). Singh et al. (2009), also obtained one highest productivity (42.2 t $ha^{-1}$) by applying 100% of the crop evapotranspiration, based on the class A pan evaporation.

Analyzing the yield of tomato resulting from $CO_2$ injection and six irrigation depths, Carraro e Duarte (2002) observed that the maximum yield of fruit, without $CO_2$ application, was obtained by applying the irrigation depth of 335.2 mm. Applying the irrigation depth corresponding to 100% of the ETc in tomato, Etissa et al. (2014) obtained a yield of 82.14 t $ha^{-1}$.

It was observed an increasing of the percentage of rotten fruits upon the increasing of the irrigation depths. The highest percentage of rotten fruits (5.46%) corresponded to the highest irrigation depth (650 mm).

Analyzing the yield of tomato as a function of $CO_2$ injection and six irrigation depths, Carraro and Duarte (2002), observed that the maximum yield of fruit, without $CO_2$ application, was obtained by applying the irrigation depth of 335.2 mm.

The results show that the application of higher irrigations depths resulted in higher productivity. This increasing in productivity associated with the increase in the irrigation depths can be related to the reduction of abortion of flowers and fruits, result in in lower abortion of fruits and higher pollen viability in the flowering and beginning of the fruiting stage. However, in the water deficit condition, there was a decrease in size and number of fruits per plant, which compromised the fruit yield (Marouelli and Silva, 2006).

Marouelli et al. (2012) elucidated that the occurrence of water deficit, even when moderate, reduces fruit size, compromising productivity. The occurrence of severe water deficit can reduce pollen viability and the number of fruits per plant, besides causing physiological damage, such as blossom-end rot.

The rotting of fruits might have resulted from a higher exposure of fruits to the wet surface of soil and was aggravated by the use of sprinkler irrigation. The rotting of fruits might also have been aggravated due a higher vegetative growing of the aerial part, which developed in an environment with higher humidity between plants. Under these conditions, the environment may have favored the appearance of fungal and bacterial diseases such as *Rhizoctonia* and soft rot, respectively (Marouelli et al., 2007).

The pulp yield linearly increased by the increasing of irrigation depths and the largest yield (13.1 t $ha^{-1}$) was obtained by applying the irrigation depth of 522 mm (Figure 5). As to the soluble solids, the increasing of irrigation depth resulted in a quadratic decrease of these compounds. The higher value of °Brix (4.2 °Brix) was obtained with the irrigation depth of 446 mm. Sezen et al. (2010) obtained the highest value of TSSC (4.38% Brix) by applying 100% of the class A pan evaporation.

The TSSC of fruits is directly influenced by climatic conditions, genetic constitution, maturation stage (Javanmardi and Kubota, 2006) and irrigation (Dumas et al., 2003). A high availability of water to plants during the ripening stage may have a negative effect on TSSC of

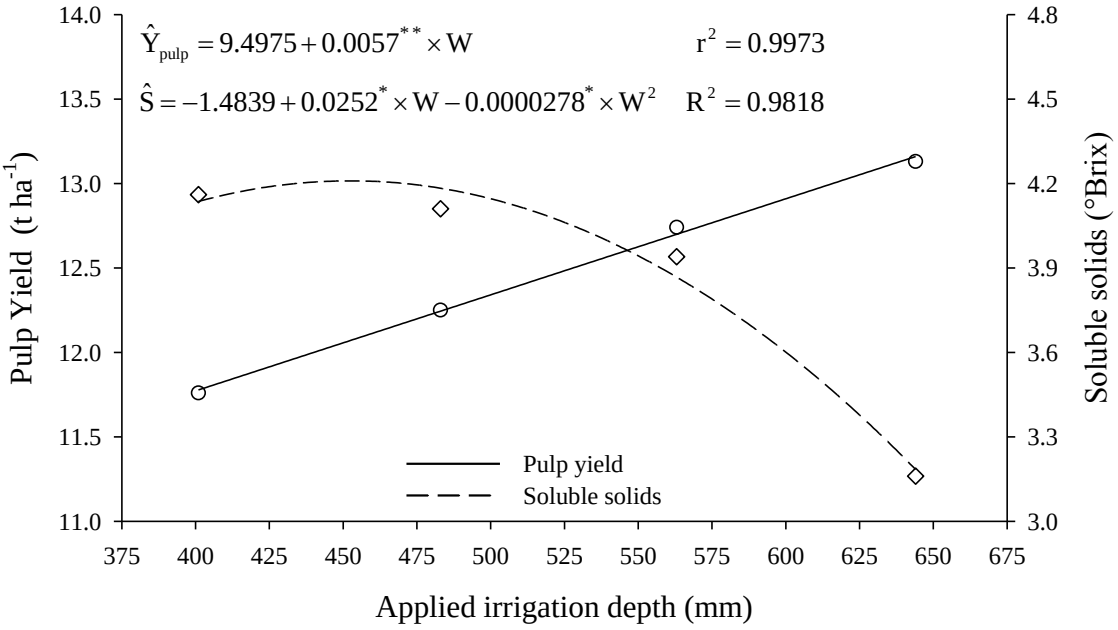


**Figure 5.** Pulp yield and soluble solids as a result of the different irrigation depths applied. *Significant level of 5% probability by Student "t" test.

**Table 3.** Means of production for the different treatments of suspension of irrigation (S1, S2 and S3).

| Production variables | Treatments | | |
|---|---|---|---|
| | S1 | S2 | S3 |
| Yield (t ha$^{-1}$)* | 89.1$^{b}$ | 89.0$^{b}$ | 103.7$^{a}$ |
| Percentage of rotten fruits (%)** | 2.7$^{a}$ | 4.0$^{a}$ | 2.9$^{a}$ |
| Pulp yield (t ha$^{-1}$)** | 12.2$^{a}$ | 12.0$^{a}$ | 12.9$^{a}$ |
| Soluble solids (°Brix)** | 4.2$^{a}$ | 4.2$^{a}$ | 4.0$^{b}$ |

The averages followed by the same letter do not differ by Tukey test at the probability of *a 5% and **a 10%.

fruits. A higher availability of water during the ripening stage can reduce the amount of sugars in fruits, increasing the cost of their dehydration during the production of tomato paste (Hanson et al., 2006).

As shown in Table 3, the fruit yield was statistically higher for S3 treatment (103.7 t ha$^{-1}$). The other treatments did not differ significantly. On the other hand, the TSSC was statistically higher for the S1 and S2 treatments, both with a value of 4.2 ° Brix, differing from the S3 treatment.

This trend can be explained by the greater water availability to plants during their cycle. These factors resulted in the production of bigger fruits and lower losses due the abortion of flowers and fruits, resulting in higher productivities (Pires et al., 2009).

The management of irrigation suspension should be caried out at the proper time in order to obtain a good row material for processing (Cahn et al., 2002). The lower TSSC obtained from the S3 treatment results from the application of irrigation during almost the entire crop cycle.

## Conclusions

The irrigation depth of 84.5% resulted in the highest productivity of ripe fruits. The suspension of irrigation at 118 days after transplanting and 5 days before the harvest provided the highest productivity of fruits and the highest soluble solids content, providing higher quality and production of fruits to tomato industry.

## Conflict of Interests

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Prevalence of viruses infecting plantain (*Musa* sp., AAB genome) in the major growing regions in Cote d'Ivoire

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**This study was undertaken to determine the prevalence of viruses, viz Banana streak Obino l'ewai virus (BSOLV), Cucumber mosaic virus (CMV), Banana mild mosaic virus (BanMMV) and Banana bract mosaic virus (BBrMV) causing serious diseases on plantain in Cote d'Ivoire. From April 2010 to July 2010 and June 2011 to August 2011, 103 farmers' plots located in 13 important plantain productions regions were screened. In all, 424 samples having symptoms of yellow or moderate chlorotic streaks were analyzed by reverse transcriptase-PCR and by immunocapture-PCR. Viruses identified were BSOLV, BanMMV and CMV respectively in proportions of 78%, 63% and 5.4% of the samples analyzed. Mixed infections of these three viruses were found in the 13 regions while CMV was present only in 3 regions. None of the samples collected were infected by BBrMV. Infected suckers used by farmers to establish their banana field could be the cause of these viral infections. The results showed that 9% of symptomatic samples were not associated with the presence of one or the other of the viruses studied. Further study is required to identify reported viruses in banana and plantain across the world.**

**Key words:** Plantain, farming modes, viruses, reverse transcription polymerase chain reaction (RT-PCR), immunocapture polymerase chain reaction (IC-PCR), Cote d'Ivoire.

## INTRODUCTION

Plantain is a staple food for millions of people in many countries of West and Central Africa and an important source of income for producers (Lescot and Ganry, 2010). According to FAO (2012), plantain is the third food crop produced in Cote d'Ivoire after yam and cassava. Indeed, originally grown around homes, plantain has, for decades, greatly expanded because of its association with food and/or industrial crops (Traore et al., 2009). The different cultivars of bananas, including plantains, whatever the areas and production methods, are affected

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by a wide range of pests and diseases (Ploetz, 2004). Viral diseases play a destructive role reducing the levels of production and the quality of products in both industrial and village plantations and limiting the exchanges of germplasm for improvement programs (Geering, 2007). The main viruses natura lly infecting banana are Banana bunchy top virus (BBTV, *Babuvirus, Nanoviridae*), Cucumber mosaic virus (CMV, *Cucumovirus*, *Bromoviridae*), several species of Banana streak virus (BSVs, *Badnavirus*, *Caulimoviridae*), Banana bract mosaic virus (BBrMV, *Potyviridae*) and Banana mild mosaic virus (BanMMV, Flexiviridae). The symptoms caused by BBTV on banana, consisting of a marginal chlorosis and bunching of leaves at the top of the pseudostem, forming a rosette with a "bunchy top" appearance are very characteristic (Su et al., 2003). On the other hand, for the other viruses the visual diagnosis is not enough because the symptoms are not specific (Iskra-Caruana et al., 2008).

In addition, during mixed infections between BanMMV and BSVs or CMV, often encountered in plantation, symptoms of BanMMV are masked by those caused by other viruses (Lockhart, 2002; Reichel et al., 2003). Banana bunchy top virus, BSVs and BanMMV are associated with the genus *Musa* (Kumar et al., 2014) while CMV has an extremely wide host range with over 1,200 plant species representing 100 plant families (Jacquemond, 2012). Banana bract mosaic virus known for a long time to have been associated with the genus *Musa* has been reported for the first time on cardamom plants (*Elettaria cardamomum* Maton), an herbaceous monocotyledonous rhizome of the *Zingiberaceae* family, in India (Siljo et al., 2012). Among the 5 viruses, only 4 (BBTV, BSVs, BanMMV, CMV) have been reported in Africa (Pietersen and Thomas, 2005), BBrMV being limited in some Asian countries has been detected in Colombia and Ecuador in Latin America (Alarcon et al., 2006, cited by Quito-Avila et al., 2013 ; Quito-Avila et al., 2013). The spread of these viruses takes place, according to each species, either by insect vectors from primary foci of infection, or through the use of propagation materials already infected or by agricultural tools for CMV (Pietersen and Thomas, 2005).

However, an original mode of transmission, the vertical transmission from integrated functional viral sequences of BSVs (endogenous BSVs) in the genome of bananas of the species *M. balbisiana* (B genome) was also highlighted by several authors including Geering et al. (2005) and Cote et al. (2010). This phenomenon called viral activation leads to the generation of infectious virions responsible of streak symptoms on banana and may be due to abiotic and biotic stress action (in vitro culture, interspecific crosses) (Dallot et al., 2001; Lheureux et al., 2003; Cote et al., 2010). Banana streak virus is therefore considered the most atypical virus among the major five viruses infecting banana. Indeed, this disease known as streak mosaic may be the result of a complex of species of BSVs. Eleven distinct species of free BSVs are known such as Banana streak Obino l'ewai virus (BSOLV), Banana streak gold finger virus (BSGFV), Banana streak mysore virus (BSMYV), Banana streak imove virus (BSIMV) and Banana streak cavendish virus (BSCAV) (James et al., 2011; Stainton et al., 2015). As for BanMMV, very few studies are available on this virus and namely on its spreads; for now the only known mode of transmission is the plant material (Teycheney et al., 2005). On the other hand, BanMMV seems to have no impact on the host plant in terms of morphology and yield when it is in simple infection but it is important in co-infection (Caruana, 2003).

Cucumber mosaic virus and BSV affect bananas (Cavendish, AAA genome) in the fields of industrial exploitation for more than three decades in Cote d'Ivoire (Lassoudière, 1979; Aka et al., 2009). The works carried out on the economic impacts of BSV showed that yield losses could reach 70% after a growth cycle on the cultivar Poyo (Cavendish, AAA genome) in the location of Azaguié (Lassoudière, 1979). On plantain (AAB genome), the only study by Kouadio et al. (2013) reported for the first time the presence of BanMMV. However, to date no study has been conducted to determine the prevalence and geographical distribution of the three viruses already identified as well as those known worldwide in the production areas on plantains (AAB genome) in the country. A thorough knowledge of the viruses infecting plantains based on farming mode is therefore necessary for the development of control methods. Such measures include the strengthening of quarantine and an appropriate management of plant genetic resources. The aim of this study is to determine the prevalence of the major viruses infecting plantains in the main producing regions in Cote d'Ivoire.

## MATERIALS AND METHODS

### Surveys and collection of samples

Surveys were conducted from April 2010 to July 2010 and June 2011 to August 2011. Hundred and three farmers' plots located in 13 regions (South, Southeast, East, center and center-west) of Cote d'Ivoire were covered (Figure 1). The choice of collection areas was based on their importance for the production of plantains (Koffi, 2007). Generally, 4 to 10 samples were collected by plot depending on the size and distribution of the disease.

The sample selection was based on the presence of viral symptoms (Figure 2) and in total 424 plantain samples were collected in farmers' plots. During the surveys an inventory of plants grown in association with plantain was also achieved by taking a census of plants grown in plots visited. In addition, information on the plantain cultivar, the health status of planting material as well as its origin was asked to the producer. We also recorded the location of plantain plants showing symptoms of virus diseases from the sampling plot. On the collection sites, the samples were packed in plastic bags with a unique code and stored on ice during their transportation to the laboratory where the samples were preserved by drying over calcium chloride ($CaCl_2$) and kept at 4°C for molecular analysis.

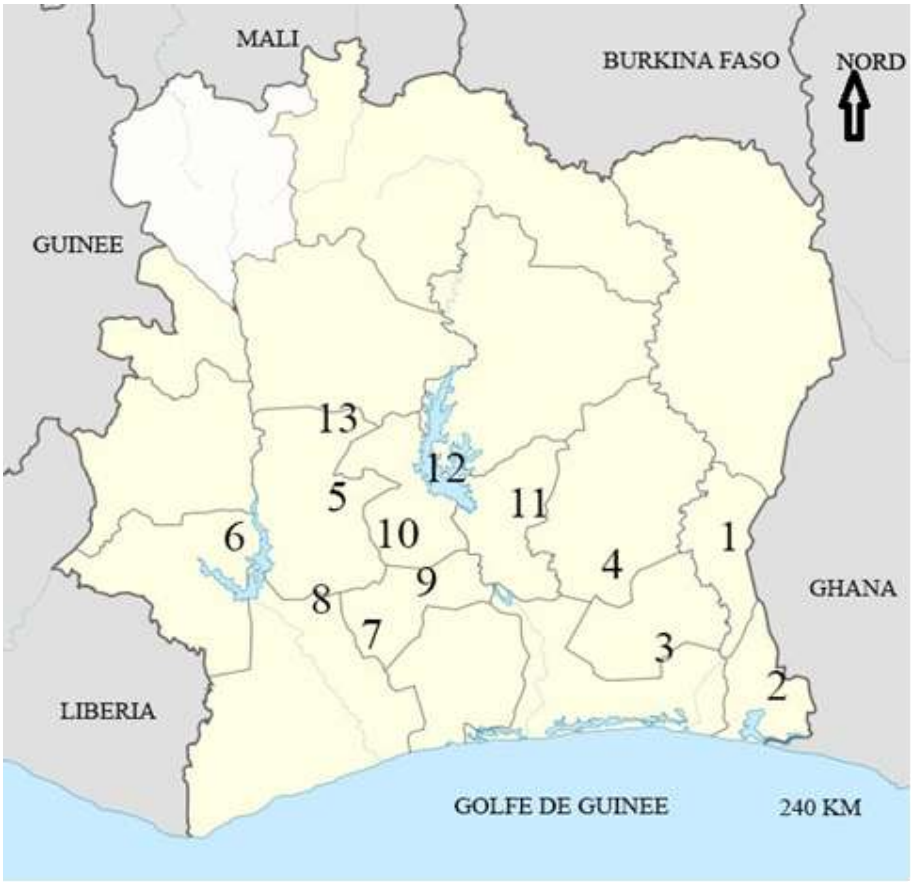


**Figure 1.** Map of Cote d'Ivoire showing the location of regions 1 to 13 where field grown banana plantain crops were surveyed for mosaic virus diseases in 2010 and 2011: 1: Abengourou; 2: Aboisso; 3: Agboville; 4: Bongouanou, 5: Bouaflé; 6: Daloa; 7: Gagnoa; 8: Issia; 9: Oumé; 10: Sinfra; 11: Toumodi; 12: Yamoussoukro; 13: Zuénoula

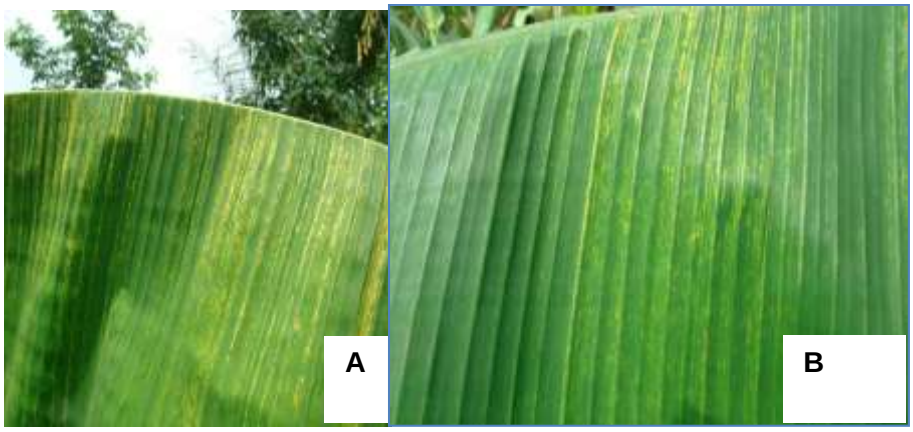


**Figure 2.** Yellow and/or chlorotic streak symptoms on 2 plantain samples collected in Cote d'Ivoire: A : sample Y26 collected in Yamoussoukro ; B : Sample GB9 collected in Agboville.

**Molecular detection of CMV, BanMMV and BBrMV by reverse transcriptase-polymerase chain reaction (RT-PCR)**

Molecular detections by RT-PCR were performed to detect the presence of CMV, BanMMV and BBrMV in the plantain samples collected. The primers used come from the genes encoding the coat proteins of these viruses (Table 1). The samples were put into a grinding bag in the ratio of 0.5 g of fresh leaves for 2 ml (control)

**Table 1.** Primer sequences and target templates.

| Primer name | Primer sequence (5'-3') | Target virus | Reference |
|---|---|---|---|
| BanCP1 | GGATCCCGGGTTTTTTTTTTTTTTTTV | BanMMV | Teycheney et al., 2005 |
| BanCP2 | TATGCNGCNTTYGAYTTCTTRGAYG | BanMMV | Teycheney et al., 2005 |
| BractN2 | ACATGGAGTATGATGGATAAGG | BBrMV | Iskra-Caruana et al., 2008 |
| BractNR | GTGTGCYTCTCTAGCCCT GTT | BBrMV | Iskra-Caruana et al., 2008 |
| CMV3' | TTTTAGCCGTAAGCTGGATGGACAACCC | CMV | Sharman et al., 2000 |
| CMV5' | TATGATAAGAAGCTTGTTTCGCGCA | CMV | Sharman et al., 2000 |
| BSV cl1 | ATGGCCTTAATAGTCTTTCGTGAT | BSOLV | Dallot et al. 2001 |
| BSV cl2 | GGTGGCGCTGAG GATGTG | BSOLV | Dallot et al. 2001 |

or 50 mg of dried leaves for 1 ml of extraction buffer at pH 7.2 (137 mM NaCl; 8 mM $Na_2HPO_4$; 1.5 mM $KH_2PO_4$; 2.7 mM KCl; 80 mM $Na_2SO_3$; 3 mM $NaN_3$, 0.05% Tween 20), the dried leaves being previously soaked for 15 min in the extraction buffer. Grinding using an electric grinder (Power Plus X022) was then performed. The juice clarified by decantation (maintained on ice) was collected in Eppendorf tubes before being diluted 100 times with sterile distilled water for the RT-PCR reaction. The reaction was performed in a volume of 25 µl (20 µl of reaction mixture + 5 µl of the sample) by using the Titan RT-PCR kit (Rock, Mannheim, Germany). The reaction mixture consisted of 5 µl of Buffer (5× Concentrate) Titan RT-PCR, 0.5 µl of dNTP, 0.5 µl of each primer at 25 µM, 1.25 µl of DTT (100mM), 0.5 µl of enzyme mix Titan, and 11.75 µl of sterile distilled water. All amplification reactions included negative and positive controls from banana plants grown in the greenhouse as well as blanks made of sterile distilled water. The amplification was performed in a thermocycler (My Cycler$^{TM}$, Biorad, USA) according to the following program: 50°C for 30 min for the reverse transcription, 94°C for 5 min for the first denaturation followed by 40 cycles (94°C for 30 s for the second and the following denaturations; 54°C for 1 min for annealing; 72°C for 2 min for elongation) and final elongation at 72°C for 10 min. The last step was the revelation of amplification products on agarose gel 1% [1 g agarose for 100 ml of Tris Acetate EDTA (TAE) 1 x buffer] containing 10 µl of ethidium bromide in buffer TAE 1×. The electrophoretic migration took place under a constant current of 120 mA for 45 min. The gel was then visualized under ultra violet light allowing the observation of amplified bands. It is important to clarify that there was no simultaneous detection; the collected samples have been separated for indexing each of the three viruses BanMMV, BBrMV and CMV.

#### Detection of BSOLV by immunocapture (IC)-PCR

The detection of BSOLV was performed by IC-PCR using a specific polyclonal antiserum (SEDIAG, France) and the BSV cl1 and BSV cl2 primers targeting the gene encoding the viral capsid protein and detecting the species BSOLV (Table 1). The difficulty to detect exogenous forms of BSV species makes the IC-PCR a commonly used technique for detection of that virus (Agindotan et al., 2006; Geering et al., 2011). The IC-PCR was performed following the protocol described by Dallot et al. (2001).

## RESULTS

### Analysis of cropping systems of plantain

Following the surveys carried out in 2010-2011 in 103 plots located in the 13 regions, there are three major farming systems of plantain: crops around homes, monoculture and the associated cropping with 3 types. The types of this associated cropping are the association with other food crops, the association with industrial crops and the association with other food and industrial crops (Table 2). The associated cropping system of plantain is the most common with a proportion of 81.5% of all plots visited. Only 8.7% and 9.7% of the farmers respectively practice crops around their homes and monoculture (without association with other crops). These farmers are located in the regions of Abengourou, Aboisso, Bouafle, Gagnoa, Toumodi, Yamoussoukro and Zuénoula. The industrial crop associated with the plantain is cocoa followed by the rubber tree. Among the food crops we have cocoyam (*Xanthosoma sagitifolium*) and corn that have been observed in the majority of plots visited in the 13 regions. Other food crops include pepper, eggplant and yam. Regarding the type of planting material, farmers use local cultivars of "French" and "False-Horn" plantain. Of the 424 samples collected, 82 samples were taken on the cultivar "Agnrin", French plantain with 98% (74/82) coming from 3 regions (Abengourou, Bongouanou and Aboisso). The 342 samples of the cultivar "afoto" False-Horn Plantain come from plots located in the 10 other regions. In addition, in all plots visited, the material of new plantations consists of suckers from old plantations without prior phytosanitary indexing. During surveys, the banana plants were at different ages including bananas less than a year and bananas more than one crop planting cycle.

### Overall prevalence of viruses in the 13 regions visited

The results of the identification of the viruses in the 424 samples collected in 66 plots plantains with symptomatic plants over the 103 visited are summarized in Table 3. The specific primers used for the molecular detection of these viruses generated DNA fragments about 500bp, 500bp, 350bp and 300 bp respectively for CMV, BSOLV, BanMMV and BBrMV (Figure 3). Expected sizes of the

**Table 2.** Percentage of presence by region visited of the different cultivation systems including plantain in farmers' fields in Cote d'Ivoire between 2010 and 2011

| Locations | Number of plots visited | Crops around homes (%) | Monoculture (%) | Industrial crops (%) | Food crops (%) | Industrial and food crops (%) |
|---|---|---|---|---|---|---|
| Abengourou | 5 | 20 | 20 | 0 | 20 | 40 |
| Aboisso | 7 | 28.5 | 0 | 0 | 57.1 | 14.2 |
| Agboville | 9 | 0 | 11 | 0 | 33.3 | 55.5 |
| Bongouanou | 7 | 0 | 14.2 | 0 | 0 | 85.7 |
| Bouaflé | 14 | 7.1 | 7.1 | 42.8 | 21.4 | 21.4 |
| Daloa | 4 | 0 | 25 | 50 | 25 | 0 |
| Gagnoa | 10 | 20 | 20 | 10 | 50 | 0 |
| Issia | 6 | 0 | 0 | 0 | 33.3 | 66.6 |
| Oumé | 4 | 0 | 0 | 0 | 25 | 75 |
| Sinfra | 10 | 0 | 0 | 10 | 10 | 80 |
| Toumodi | 8 | 12.5 | 12.5 | 0 | 25 | 50 |
| Yamoussoukro | 13 | 7.7 | 15.3 | 0 | 76.9 | 0 |
| Zuénoula | 6 | 16.6 | 0 | 0 | 33.3 | 50 |
| **Total** | **103** | **8.7** | **9.7** | **9.7** | **33.9** | **37.9** |

amplicons were observed in all positive controls. On the other hand, no amplification was observed for negative controls (healthy plants) and the blanks. In all, 91% (386/424) of the samples collected were found to be infected with at least one of the four viruses in this study (Table 3). The viruses identified were BSOLV, BanMMV and CMV respectively in the proportions of 78%, 63% and 5.4% of the samples analyzed. None of the collected survey samples was positive for BBrMV. BSOLV and BanMMV dominated either single or mixed infections. Single infections accounted for 39% of the samples tested (that is 167 over 424) of which 115 samples infected with BSOLV (27%) and 52 samples infected with BanMMV (12%). As for mixed infections BSOLV + BanMMV, they were around 46% of the samples analyzed. A double infection BSOLV + CMV was observed in one of the locations (Bongouanou) whereas no mixed infection BanMMV + CMV was identified. A triple infection BSOLV + BanMMV + CMV was observed in 4.7% of the samples collected. BSOLV and BanMMV were detected in the 13 regions visited while CMV was positively indexed in the samples of 3 regions [Bouaflé (14/89), Gagnoa (3/38) and Bongouanou (6/45)]. In all, the percentages of symptomatic samples infected with at least one of the four viruses studied are above 79% in all areas of collection (Table 3) including a value of 100% in the locations of Abengourou and Daloa. Although BSOLV and BanMMV were present in all the locations visited, a difference of prevalence was nevertheless observed in the samples collected (Figure 4). Thus, BSOLV was the most present virus in 9 of the 13 locations visited, with high proportions in the locations of Zuénoula (93%), Bongouanou (93%), Abengourou (86%), Issia (84%) and Bouaflé (84%) while BanMMV was dominant in the locations of Sinfra (76.7%), Gagnoa (76%) and Oumé (69%). The single BSOLV infections were higher in the locations of Abengourou (52%), Issia (50%), Agboville (45%) Aboisso (45%) and Yamoussoukro (38%).The percentages of the most important and prevailing single infections due to BanMMV in our samples were 28% and 26% respectively in Gagnoa and Oumé. The mixed infections BSOLV + BanMMV ranged from 21% (Aboisso) to 80% (Toumodi).

## Prevalence of virus based on plantain cultivars and cropping systems

From the two cultivars of plantain subgroup observed in the plots visited, the proportion of infections due to BSOLV were 78% (64/82) and 79% (270/342) respectively for the types of cultivars French and False-Horn in the samples analyzed. Similarly, BanMMV was present in 58% (48/82) and 64% (220/342) of the cultivars French and False-Horns plantains analyzed. CMV was detected in 4% (3/82) of the samples of the cultivar type French and 6% (20/342) of the samples of cultivar type False-Horns. These results show that there are few differences in the proportions of viral infections among the plantain cultivars.

The analysis of the frequency of viral infections in the samples tested, based on cropping systems including plantain reveals a high frequency of BSOLV followed by BanMMV (Figure 5). In at least 56% of cases, the observation of the chlorotic streaks symptoms was explained by the presence of BSOLV. We can note that this frequency of BSOLV was higher (81%) in the associations of plantain with other food and industrial crops although it was present in other cropping systems. This trend is similar for BanMMV where the frequency

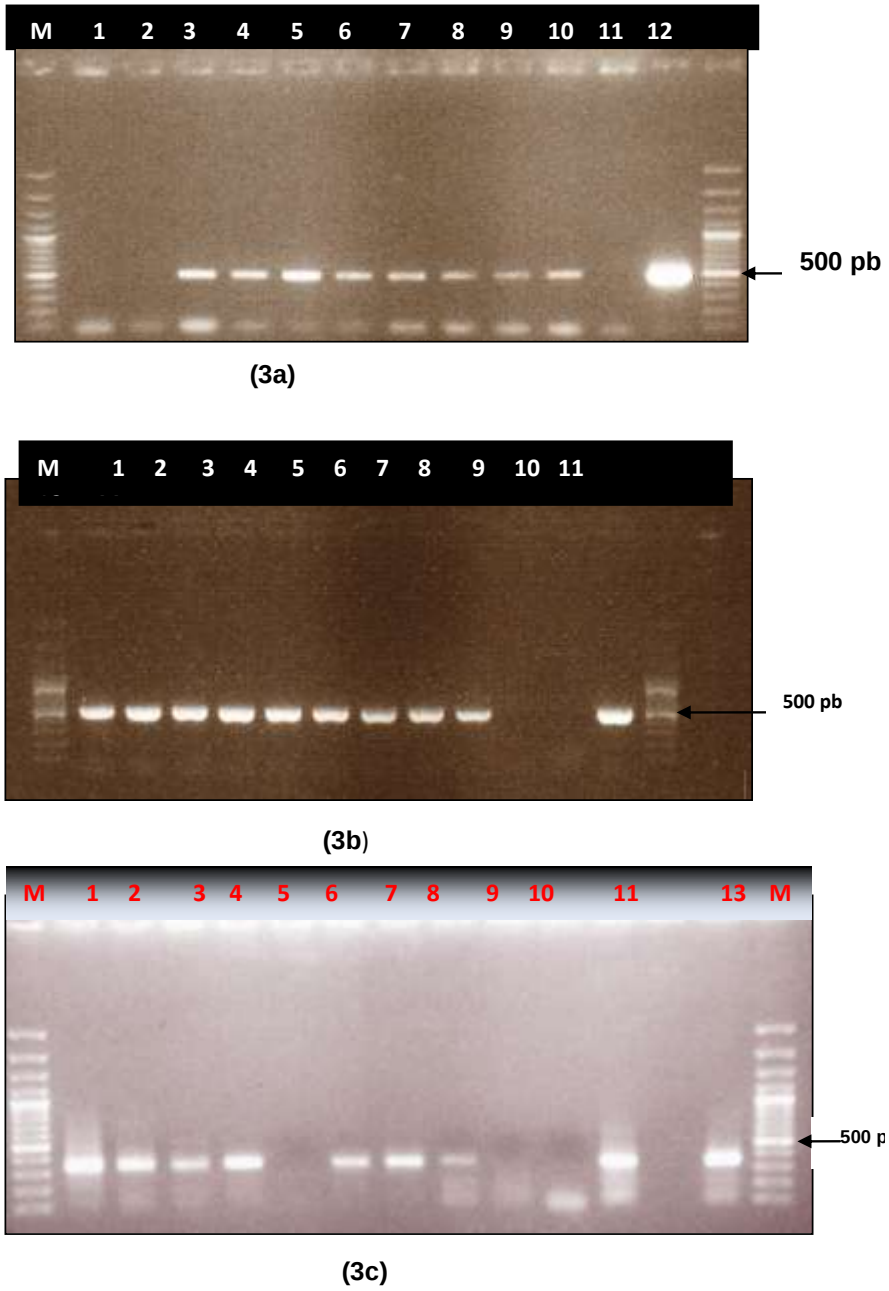


**Figure 3.** (**a**) Molecular detection of BSOLV by IC-PCR in samples of plantains collected in Cote d'Ivoire, M: Marker of molecular weight of 100 bp (Ladder DNA), 1-2: Healthy plant in the greenhouse; 3-10: Amplicons (500 bp) of 8 samples of banana; 11: Mix + sterile distilled water; 12: Positive control BSOLV; (b) (1%) agarose gel electrophoresis of the amplification products obtained by reverse-transcriptase (RT) -PCR for the detection of CMV: M: Marker of molecular weight 100 bp (DNA Ladder), 1-9: Amplicons of 500 bp in 9 symptomatic banana samples; 10: Mix + sterile distilled water, 11 healthy plant in the greenhouse, 12: Positive control CMV; (c) (1%) agarose gelelectrophoresis of amplification products obtained by RT-PCR for detection of BanMMV: M: Marker of molecular weight 100 bp (DNA Ladder); wells 1-8: Samples of plantains collected; wells 9-10: Healthy plant and blank; 11 and 13 positive controls for BanMMV

was 70% in the associations of plantain with food and industrial crops. The presence of CMV remains relatively low but was higher in crop around homes (22%) and crop associated with food crops including peppers, eggplant. CMV was not detected on plantain associated with cocoa, coffee and rubber.

**Table 3.** Occurrence of Banana streak Obino l'ewai virus (BSOLV), Banana mild mosaic virus (BanMMV), Cucumber osaic virus (CMV) and Banana bract mosaic virus (BBrMV) on plantain leaf samples with virus-like symptoms collected from farmers' fields surveyed during 2010-2011 in Cote d'Ivoire.

| Location | Fields[a] | No. Analyzed | BSOLV | CMV | BanMMV | BBrMV | Positive total |
|---|---|---|---|---|---|---|---|
| Abengourou | 5/5 | 23 | 20 | 0 | 11 | 0 | 23 (100)[b] |
| Aboisso | 6/7 | 29 | 19 | 0 | 10 | 0 | 23 (79) |
| Agboville | 5/9 | 20 | 14 | 0 | 7 | 0 | 16 (80) |
| Bongouanou | 5/7 | 45 | 42 | 6 | 34 | 0 | 44 (98) |
| Bouafle | 8/14 | 89 | 75 | 14 | 69 | 0 | 85 (95) |
| Daloa | 1/4 | 10 | 8 | 0 | 7 | 0 | 10 (100) |
| Gagnoa | 9/10 | 38 | 24 | 3 | 29 | 0 | 35 (92) |
| Issia | 5/6 | 26 | 22 | 0 | 10 | 0 | 23 (88) |
| Oume | 4/4 | 26 | 16 | 0 | 18 | 0 | 23 (88) |
| Sinfra | 4/10 | 30 | 22 | 0 | 23 | 0 | 28 (93) |
| Toumodi | 1/8 | 5 | 4 | 0 | 4 | 0 | 4 (80) |
| Yamoussoukro | 9/13 | 52 | 39 | 0 | 22 | 0 | 42 (81) |
| Zuenoula | 4/6 | 31 | 29 | 0 | 24 | 0 | 30 (97) |
| Total | 66/103 | 424 | 334 (78) | 23 (5.4) | 268 (68) | 0 | 386 (91) |

[a]Fields with symptomatic plants/fields surveyed; [b]In parentheses are percentages calculated over the total number of virus-infected plants. No, Number of samples.

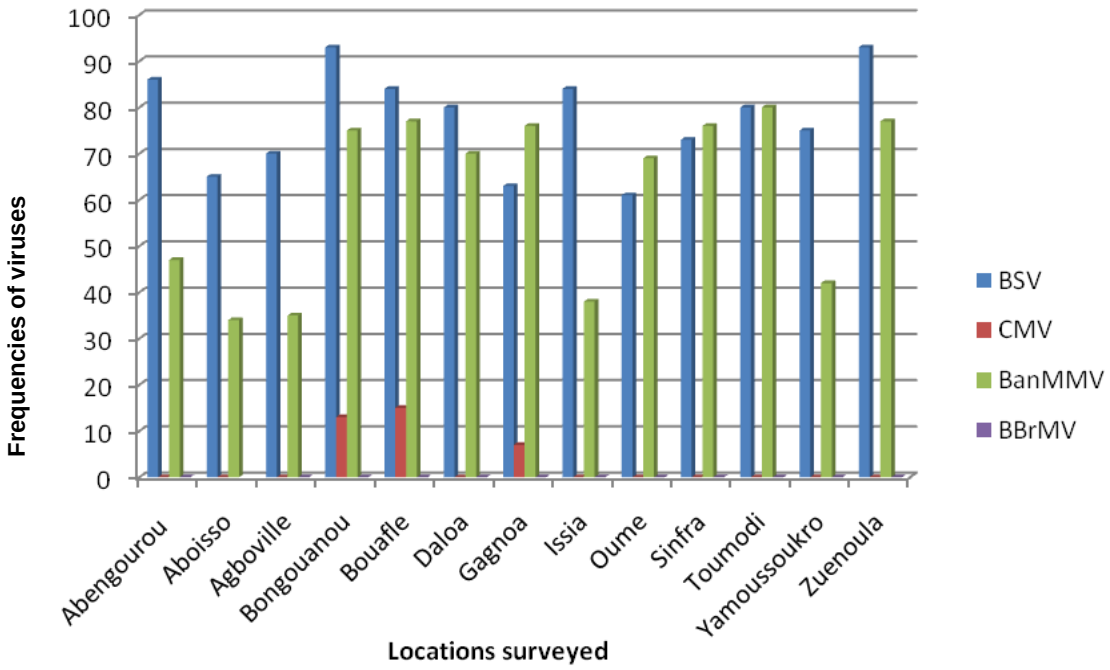


**Figure 4.** Frequencies of viruses identified in locations visited during field surveys in main plantain growing areas in Cote d'Ivoire.

## DISCUSSION

The results of the molecular tests carried out on symptomatic samples of plantain collected in 2010-2011 in Cote d'Ivoire have shown that these plants are infected by three viruses over the four tested. In addition, in the 13 regions visited, the viruses identified could infect local cultivars of plantains namely French and False-Horn plantains. Viruses were mentioned on plantains by Kouadio et al. (2013) in Cote d'Ivoire, but this study provides some missing information to date on the prevalence of the viruses in the main production areas in

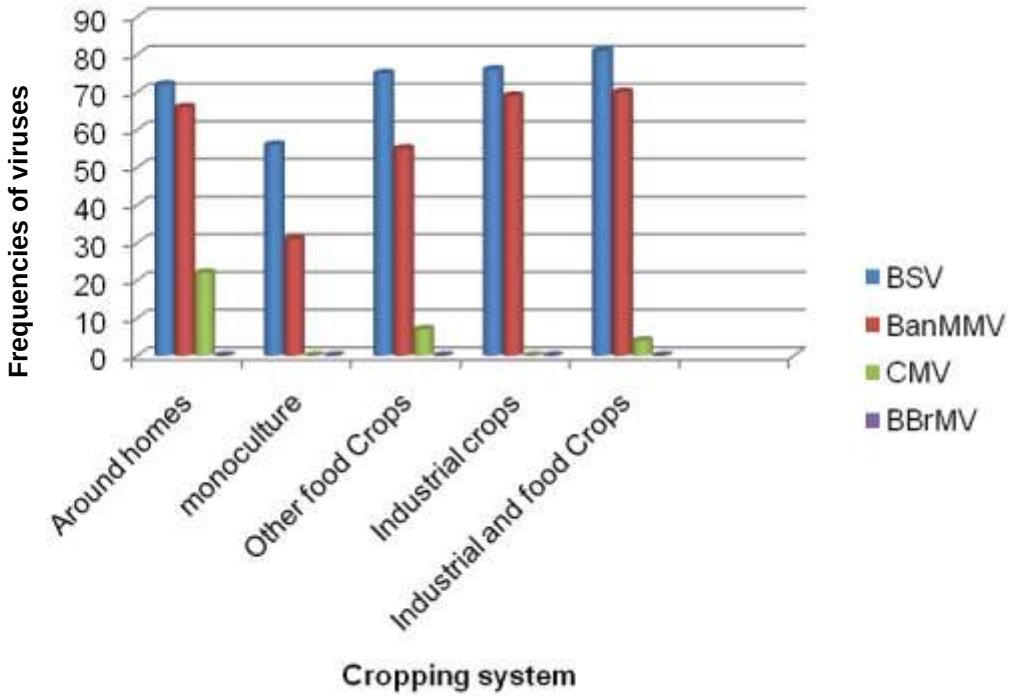


**Figure 5.** Frequencies of viruses identified according to plantain cropping systems.

relation to cropping systems including plantain. The results showed a predominance of BSOLV and BanMMV in the 13 regions visited and in different cropping systems including plantain. In Africa BSOLV was reported on plantain in several countries such as Benin, Nigeria, Ghana (Kumar et al., 2014). According to Caruana (2003), BanMMV has a worldwide distribution. This virus has indeed been detected in samples coming from Africa, America, Asia and Australia (Caruana, 2003). The high prevalence of BSOLV followed by BanMMV in the main production areas of plantain in Cote d'Ivoire suggests a viral transmission due to contaminated planting material through the transfers of plant material between producers.

In fact, the surveys have shown that farmers use suckers that have not been indexed and certified coming from old plantations. In addition, the symptomatic plants showed a random distribution in the plots of plantain visited. BSOLV can be transmitted through infected suckers (Kengamal et al., 2008; Kumar et al, 2014), although the infection by the activation of the viral sequences integrated in the genome of the species *M.balbisiana* can also take place under some conditions of stress such as in vitro culture (Cote et al., 2010) or a longer drought (Hauser, 2010). Plantains (AAB genome) grown in Cote d'Ivoire are not derived from *in vitro* culture but rather from suckers as mentioned in our surveys and already shown by Traoré et al. (2009). Similarly, the collection of samples was conducted during the rainy seasons in the areas subjected to a similar type of climate and located in the same agro-ecological zone, the Guinean zone. The major difference corresponded to the rainfalls between locations in the Centre (Yamoussoukro and Toumodi) and other locations with a higher amount of rainfall. Lassoudière (1979) had already shown a high prevalence of BSV on bananas (Cavendish, AAA group) in Cote d'Ivoire. The hypothesis of the transmission by infected suckers is more likely especially as the mealybug vectors are less active in the long-distance spread of BSV (Kengamal et al., 2008; Kumar et al., 2014).

However, no diagnosis via molecular markers to discriminate episomal infections from those from an activation of integrated sequences has been carried out (Kumar et al., 2014). For BanMMV, the only known mode of transmission of this virus is the infected plant material (Teycheney et al., 2005). In this study conducted in Cote d'Ivoire, in general, there were few unique infections but it was most often mixed infections of BSOLV and BanMMV. The observation of symptoms of pronounced chlorotic streaks on plantain in the collected samples could be due in part to the mixed infection between these two viruses. BanMMV could amplify the severity of symptoms caused by BSVs when it is in co-infection (Lockhart, 2002; Caruana, 2003).

However, the incidence of the viruses identified in this study could not be carried out. While waiting for the study of the impact of these viruses, these results highlight the need to develop indexing and certification tools to produce healthy planting material. Moreover, given the

introduction of new improved hybrid varieties of banana (AAB and AAAB genomes) in Cote d'Ivoire (Kouakou et al., 2012), a study on the assessment and the risk management of the activation of viral sequences of different pathogenic species of BSVs integrated into the genome of *M. balbisiana* in these interspecific hybrids should be performed. In this context of increasing plantain cropping, it would be advisable to study this diversity of species of BSVs in Cote d'Ivoire since this study took into account only one viral species. Indeed, the specific primers used for the detection of BSVs in the samples tested reveal the species BSOLV (Dallot et al., 2001).

The results also showed that CMV is present in the samples of plantains collected but with a relatively low percentage of infection (5%). The occurrence of CMV on plantain was also reported in Ghana and Nigeria (Kumar et al., 2014). In the present study, the virus was identified only in 3 regions (Gagnoa, Bongouanou, Bouaflé) and in cropping systems of plantain around homes and crops associated with food crops. Cucumber mosaic virus is also transmitted through vegetative propagation and by aphid vectors from a wide range of host plants (Jacquemond, 2012). It is the only banana virus known to date besides banana with over 1,200 host plants (Jacquemond, 2012). In Cote d'Ivoire, CMV has been mentioned on several crops including banana, pepper, eggplant, yam (Aka et al., 2009; Séka et al., 2009; Sorho et al., 2014).

However, on plantain we observed a lower proportion of infected samples. The association of plantain with industrial crops including cocoa and rubber could explain the low viral presence due to CMV. To date, no study has reported a virus infection of these crops (cocoa, rubber) with CMV. In the location of Bouaflé (center-west) where came 60% (14/23) of the samples tested positive for CMV, it was noted that cultural association in the plantain plots was dominated by pepper and around the plots with eggplants. Similarly, Estelitta et al. (1996) revealed a high occurrence of CMV in banana plantations in association with the Solanaceae like pepper in India. This could also explain the prevalence of this virus in crops around the homes (22%). Indeed, in this cropping system, some plantains are planted in the immediate vicinity of homes on garbage dumping sites. From these refuses from the kitchens, there could be the development of other plants including food crops, potential hosts of CMV that will be in the banana environment. And as among the aphid vectors *Aphis gossipii* is present in Cote d'Ivoire (Lassoudière, 2012), the transmission will be done, followed by the spread from the infected suckers.

Banana bract mosaic virus was not detected in all samples tested in this work. This is the first study that confirms the absence of the virus in Cote d'Ivoire. This suggests a strengthening of quarantine measures for the exchanges of plant material since the insect vectors including *Aphis gossipii* and *Pentalonia nigronervosa* are present in Cote d'Ivoire (Lassoudière, 2012).

Overall, 9% of the symptomatic samples collected were negatively indexed to four viruses tested. This might be due to less virus titer in the collected samples. The symptoms of chlorotic streaks could be due to other unidentified viruses or other species of BSV namely BSGFV and BSMYV (Kumar et al., 2014) not targeted in this study. Banana bunchy top virus is one of the most damaging viruses on banana (*Musa* sp.) (Kumar et al., 2011) and it is expanding in Africa including Benin and Nigeria in West Africa (Kumar et al., 2011; Lokossou et al., 2012; Adegbola et al., 2013). However, unlike the other 4 banana viruses causing symptoms including mosaics that we indexed, Banana bunchy top disease has very characteristic symptoms on banana. During surveys and sample collections no plantain plant showed such symptoms. Even if we did not observe these symptoms, one should be attentive to the extension of BBTV in Africa since the aphid vector (*Pentalonia nigronervosa)* is already present (Lassoudière, 2012).

## Conflict of Interests

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENT

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# Removal of nitrogen and phosphorus from cattle farming wastewater using constructed wetland system

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**The confined dairy cattle system employs the most modern production techniques with respect to the genetic standards of the herds. The success found in these systems promoted an increase in the number of confined animals and, consequently, an increase in the produced volume of waste, causing the waste from dairy cattle farming to be one of the largest problems in intensive management systems. This effluent has high amount of phosphorus and nitrogen, and the accumulation of these nutrients in surface waters can cause eutrophication of water courses deteriorating their quality. This study is aimed to evaluate the efficiency of a constructed wetland system, cultivated with vetiver grass (*Vetiveria zizanioides*) in the removal of important environmental pollutants nitrogen (N) and phosphorus (P) from dairy cattle farming wastewater. The effluent received pre-treatment and before passing through the constructed wetland system which was built in a trapezoidal shape. The wastewater from dairy cattle farming, at the inlet and outlet of the constructed wetland system, was biweekly analyzed for different forms of N (ammonia, total Kjeldahl N, nitrate and nitrite) and P. The constructed wetland system proposed cultivated with vetiver grass showed good removal pollutants with greater mean efficiency in the removal of nitrite (43.6%), the total Kjeldahl nitrogen (32.0%) and ammonia (31.0%). Vetiver grass cultivation showed good adaptation to the constructed wetland system, with satisfactory development and no visual symptoms of nutrients deficiency.**

**Key words:** Cultivated beds, water resources, wastes, reuse, biological treatment.

## INTRODUCTION

Agricultural activity plays an important role in the national economy, besides being one of the first economic activities to be developed in the country. With the increase in food demand, agriculture has used more

areas to intensify production.

In Brazilian agriculture, dairy farming has stood out and milk has gained space in the market, being among the most important products of this activity. The confined dairy cattle system employs the most modern production techniques regarding the genetic standards of the herds. The success found in these production systems promoted an increase in the number of confined animals and, consequently, an increase in the produced volume of waste, making it one of the largest problems in intensive management systems and a challenge for farmers and specialists, because it involves technical, sanitary and economic aspects.

Thus, the organic effluents originated from confined dairy production systems, when disposed in a receiving body, cause physical and chemical alterations in the water sources, besides posing risks to public health (Silva and Roston, 2010).

Among the technologies used in the treatment of effluents, Constructed Wetland Systems (CWSs) have stood out and shown high potential of usage, because they have been used in the treatment of effluents (Vymazal, 2011). These are systems with moderate costs of installation, reduced energy consumption and maintenance, besides landscape aesthetics.Through the use of natural processes involving vegetation, supporting medium and microorganisms, these systems are projected to be able to promote, at least partly, the treatment of effluents or other types of low-quality waters.

One of the greatest concerns in effluent treatment systems is their capacity to remove phosphorus (P) and nitrogen (N). The excessive accumulation of N in surface waters may cause eutrophication of watercourses and lead to ecological imbalance, promoting exaggerated growth of plants and animals, deteriorating the quality of the water. P, in turn, for being one of the nutrients that are essential for algae growth in watercourses, is considered as the main element involved in the process of eutrophication, which makes its removal very important in effluent treatment systems (Prochaska and Zouboulis, 2006).

Various plant species have been used and indicated for cultivation in CWSs, because they naturally occur under conditions similar to those in the CWSs. The plants used in CWSs must be perennial, with high tolerance to excess water, easy harvest and management, and high capacity to remove nutrients and pollutants (Paganini, 1997). It is noteworthy that the CWS configuration and type of substrate can influence the removal efficiency of system pollutants. Moreover, the various pollutants are not removed of the CWS in the same proportion.

Therefore, the species Vetiveria zizanioides, known as vetiver grass, very resistant to climatic variations and tolerant to contaminants (Ucker et al., 2012), is used in Brazil as vegetation on slopes, with the objective of controlling erosive processes (Andrade et al., 2011). The vetiver grass, since it has a deep and abundant root system, can be an alternative in constructed wetland systems (Barbosa and Lima, 2013).

Given the above, the aim of this study was to evaluate the capacity of removal of important environmental pollutants P and N using the constructed wetland system cultivated with vetiver grass in the post-treatment of wastewater from dairy farming.

## MATERIALS AND METHODS

### Characterization of the area

The study was carried out in the Agroecological Production Integrated System (SIPA) area, also known as “Fazendinha Agroecológica do km 47” (Figure 1), located in the municipality of Seropédica-RJ, Brazil (22º48’00’’S; 43º41’00’’W; 33 m), from June to November 2014. According to Köppen’s classification, the climate of the region is Aw, with rains concentrated from November to March, mean annual rainfall of 1,213 mm and mean annual temperature of 24.5°C (Carvalho et al., 2006).

### Wastewater treatment system

The treatment system of wastewater from dairy cattle farming (WDCF) comprised a pilot treatment unit consisting of: compost box, sedimentation tank, crushed stone filter, organic filter and constructed wetland system (CWS) with horizontal subsurface flow which was sized according to methodology presented by Marques (1999). The WDCF passed through the treatment system following the order of the previously mentioned components (Figure 2).

The CWS had a superficial area of 5.2 m² and was built in masonry and waterproofed with a 0.5-mm-thick PVC canvas; its inside was filled with crushed stone nº 1 (24 mm mesh) until the height of 40 cm and a 5-cm-thick layer of sand was in place to fix the crop (Figure 3). The CWS was built in a trapezoidal shape and its lower and upper sides were 0.5 and 1.3 m long, respectively. The hydraulic retention time (HRT) of the CWS was equal to 1.4 days (33.6 hours), considering the pore volume of the crushed stone nº 1 as equal to 50%. The wastewater from dairy cattle passed through continuously CWS.

The CWS was cultivated with vetiver grass (*Vetiveria zizanioides*), and has the following characteristics: very efficient in slope stabilization, aromatic plant that reaches height of 2 m and its roots can penetrate with a depth of 3 m (Barbosa and Lima, 2013).

Vetiver seedlings were obtained in the SIPA and planted in the CWS using a spacing of 0.12 x 0.2 m, making a total of 200 seedlings. A spacing smaller than that recommended in Andrade et al. (2011) was used in order to allow a higher number of plants for the removal of nutrients. The quantification of nutrients absorbed the culture was not carried out. The visually performance grass vetiver and average plant height after the conclusion of the experiment using measuring tape was evaluated. Were selected 20 plants randomly along the CWS. The vetiver grass height from the base to the apex of the plant was determined.

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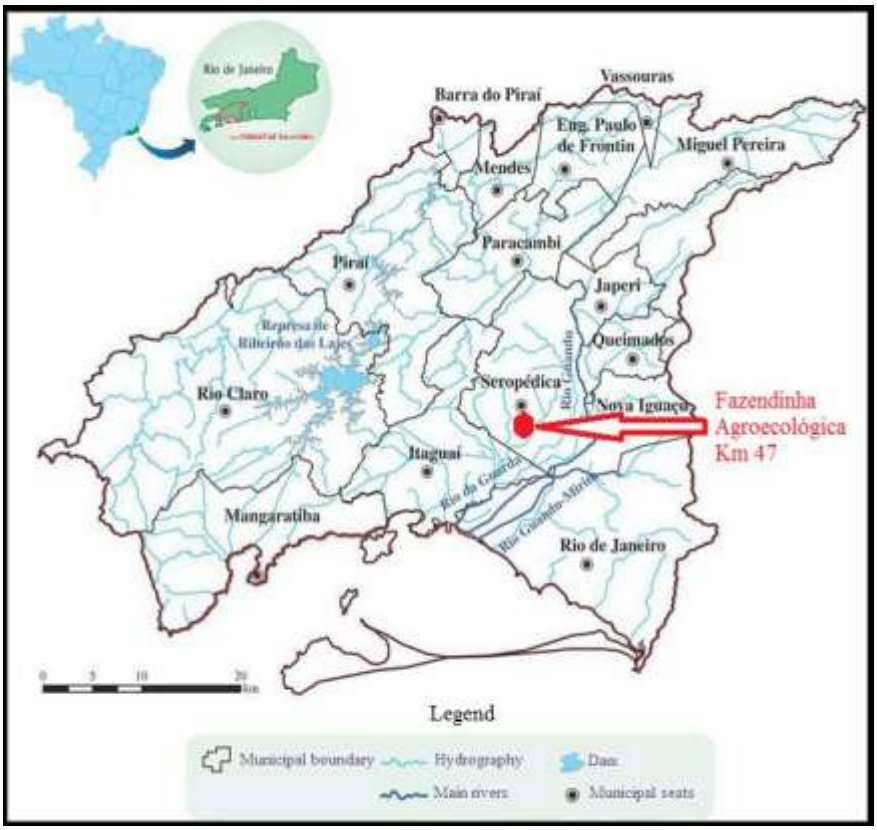


**Figure 1.** Geographic map of the study area. Source: http://www.comiteguandu.org.br/hidrografica.php (Adapted).

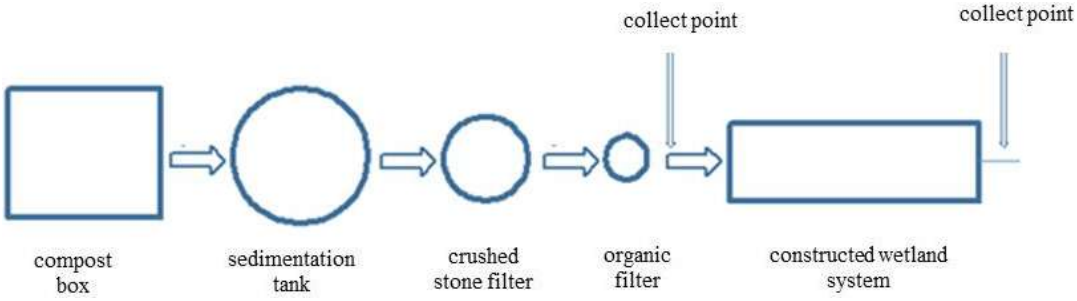


**Figure 2.** Scheme treatment system and collection points.

There are several shapes, sizes and substrates used in CWS. Therefore, it is extremely important to evaluate the efficiency of pollutant removal in the CWS proposed in this work and check if the results are consistent with those observed in the literature.

**Monitoring and evaluation of the treatment system**

The performance of the constructed wetland system was evaluated biweekly through the collection of 500 mL of WDCF at the inlet and the outlet of the CWS cultivated with vetiver. The collection of WDCF was started in August 2014. After collection, the WDCF was sent to the Laboratory of Environmental Monitoring I – Water and Effluents, of the Engineering Department in UFRRJ, for the following analyses: phosphorus, ammonia, nitrate, nitrite and total Kjeldahl nitrogen (TKN), according to the recommendations contained in APHA (1999).

The P removal efficiency of the CWS was analyzed based on the phosphate ion ($PO_4^{3-}$). It is one of the main forms of P in waters and is considered as an orthophosphate.

The main residues from dairy cattle farming are feces and urine. However, the effluent also showed water from washing, fur, milk (colostrum) and fat from the body animals and milk.

It should be pointed out that the WDCF came from the washing of the SIPA pen and there was no standardization about the amount of water used or the frequency of washings.

Before receiving the treatment, the WDCF showed mean values of 75.9, 229.9, 120.9, 46.8 and 2.2 mg $L^{-1}$ of phosphorus, ammonia,

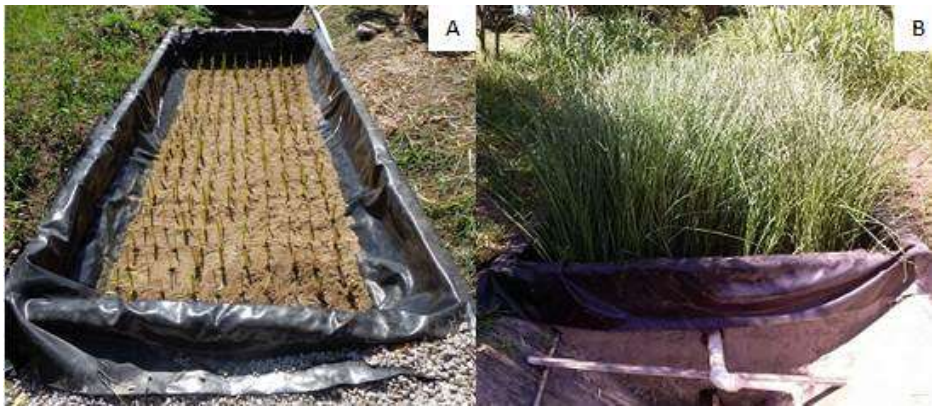


**Figure 3.** Constructed wetland system cultivated with vetiver grass; A: Transplantation of seedlings; B: Established vetiver.

**Table 1.** Inlet and outlet values and standard deviation of the analyzed parameters in the constructed wetland system cultivated with vetiver grass.

| Parameter | Inlet | Outlet | Average removal (%) |
|---|---|---|---|
| Phosphorus (mg $L^{-1}$) | 59.7 ± 26.9 | 44.1 ± 20.5 | 25.2 |
| Ammonia (mg $L^{-1}$) | 51.3 ± 12.4 | 35.4 ± 9.6 | 31.0 |
| TKN (mg $L^{-1}$) | 72.9 ± 33.4 | 49.6 ± 26.6 | 32.0 |
| Nitrate (mg $L^{-1}$) | 9.9 ± 1.3 | 8.9 ± 2.2 | 10.2 |
| Nitrite (mg $L^{-1}$) | 0.6 ± 0.2 | 0.3 ± 0.2 | 43.6 |

TKN: Total Kjeldahl nitrogen.

total Kjeldahl nitrogen (TKN), nitrate and nitrite, respectively. The mean pH value of the analyzed samples before passing through the CWS was 6.7.

## RESULTS AND DISCUSSION

The results referring to the mean values of the parameters phosphorus, ammonia, total Kjeldahl nitrogen, nitrate and nitrite at the inlet and outlet of the horizontal subsurface-flow CWS cultivated with vetiver grass are shown in Table 1.

The concentrations of all parameters analyzed in the effluent, at the CWS outlet, were lower than those at the inlet, demonstrating that there was a reduction of these parameters in the system. The reduction of pollutants has been observed in various studies with different types of wastewater using constructed wetland system cultivated or uncultivated (Tee et al., 2009; Fia et al., 2012; Chagas et al., 2011; Colares and Sandri, 2013).

The high values of standard deviation can be explained by the fact that there was no control over the wastewater that came to the SIPA's compost box, which was pumped directly into the treatment system from the cattle pen. This represented variation in the concentrations of elements and pollutants in the utilized WDCF. High values of standard deviation were also observed by Wu et al. (2014) and in various studies that used CWSs of different types for the removal of N and organic matter in wastewaters.

There were low mean concentrations of nitrate and nitrite at the outlet of the CWS cultivated with vetiver grass, which were respectively equal to 8.9 and 0.4 mg $L^{-1}$ (INEA, 2014). Nitrite is an ion that has intermediate conditions of reaction, since it is formed from the nitrification process, which is the first oxidation of ammonia in the nitrification pathway (Nunes, 2012). For this reason, it is commonly found at low concentrations in constructed wetland systems.

However, the mean concentrations of total Kjeldahl nitrogen, phosphate and ammonia in the effluents at the outlet of the horizontal subsurface-flow CWS cultivated with vetiver grass were 44.1, 49.6 and 35.4 mg $L^{-1}$, respectively. This indicates that the effluent cannot be disposed in water resources (INEA, 2014), even after passing through the treatment. Hence, potential forms of reuse of WDCF, especially agricultural reuse, must be stimulated provided that agronomic and environmental criteria are considered.

It should be pointed out that the mean concentrations of the parameters phosphorus, ammonia, total Kjeldahl nitrogen, nitrate and nitrite in the effluent at the CWS outlet were dependent on their concentrations in the

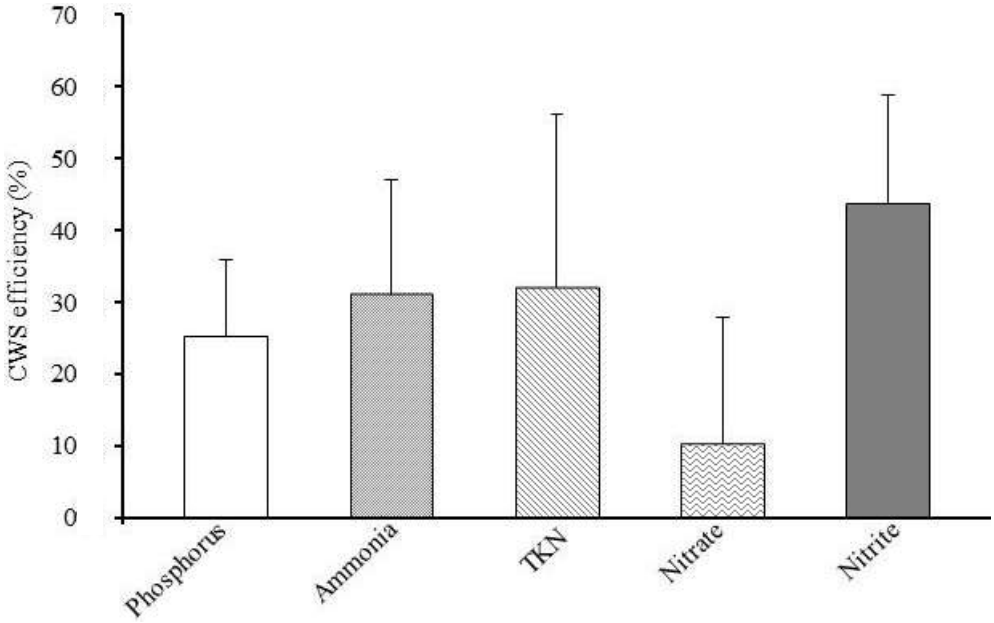


**Figure 4.** Removal efficiency and standard deviation of the parameters phosphorus, ammonia, total Kjeldahl nitrogen (TKN), nitrate and nitrite using a constructed wetland system (CWS) cultivated with vetiver grass.

WDCF at the inlet. That is, the higher the concentration of these parameters at the inlet of the CWS, the higher the concentrations at the outlet of the treatment system. No pattern of concentration for the analyzed parameters was observed at the outlet of the CWS.

Removal efficiency and standard deviation of the parameters phosphorus and nitrogen using the CWS cultivated with vetiver grass can be seen in Figure 4.

## Phosphorus

In constructed wetland systems, P removal mainly occurs through processes of adsorption, precipitation and absorption by plants (with subsequent harvest) (Vymazal, 2007).

According to the author, P removal varies between 40 and 60% among all types of wetland systems; however, results lower than 40% were found by Brasil et al. (2007), Pelissari (2013) and Stone et al. (2004). Constructed wetland systems have limited capacity for P removal, because there is no permanent loss of the nutrient in these systems, which occurs, for example, in the removal of N compounds (Ucker et al., 2012).

The mean CWS efficiency in P removal was 25.2%, which is considered good according to some literatures (Matos et al., 2010; Pelissari, 2013; Stone et al., 2004). According to Vymazal (2007), P removal in all types of CWS is low, unless special substrates with high capacity of absorption of this nutrient are used. This depends on the physical and chemical properties of the materials (Vymazal, 2014). The use of alternative substrates, such as lightweight clay aggregates and industrial slags, among others, can increase P removal efficiency in constructed wetland systems. Calcareous materials can promote the precipitation of calcium phosphate.

It should be pointed out that the filtering medium used in the experiment (crushed stone) has low retention capacity for cations to promote P adsorption (Vymazal, 2014). The observed value of efficiency was lower than those reported by Brasil et al. (2007), who obtained mean total P removal efficiency of 31 to 48%. However, it was greater than that obtained by Pelissari (2013), who observed P removal efficiency of 10% in the CWS. Most of the P is removed through the accumulation of organic P in the supporting medium and its immobilization by microorganisms (Matos et al., 2010). These authors obtained P removal efficiency of 33 to 55%, but highlighted that there are studies in the literature in which the P concentration in the wastewater increased after treatment with the CWS, i.e., there was no efficiency in the system to remove this nutrient. These authors pointed out that a possible cause for the low P removal or increase in its concentration at the outlet of the CWS is the loss of water from the system through evapotranspiration, underestimating or canceling the efficiency obtained in the CWS.

The phosphate removal efficiency showed wide variation, from 12 to 42%. Stone et al. (2004) observed P removal efficiency from 13 to 29% while working with CWSs cultivated with southern cattail for the treatment of swine farming wastewater. The release of P to the

effluent may occur due to the formation of organic acids, nitrates or sulfates in the environment, which may cause oscillations in the redox potential and reduction in pH, thus releasing P to the environment (Headley et al., 2005).

Probably, in order to obtain greater P removal efficiency, it would be necessary, among other factors, to adopt a longer hydraulic retention time (Matos et al., 2010; Vymazal, 2009; Silva and Roston, 2010) so that the effluent has a longer time of contact with the supporting medium of the treatment system and consequently improving its efficiency.

Although phosphate ion ($PO_4^{3-}$) is one of the main forms of P in water, other forms of P should be monitored in order to detect if there is removal of P in other forms available in the wastewater.

## Nitrogen

The total nitrogen expressed based on the total Kjeldahl nitrogen, corresponds to the sum of organic N, ammonia and ammonium. Hence, part of the observed removal is due to the decrease in the concentration of ammonia and mineralization of organic matter.

On average, the values of efficiency of removal of total Kjeldahl nitrogen, ammonia, nitrate and nitrite from the WDCF using the horizontal subsurface-flow CWS were 32.0, 31.0, 10.2 and 43.6%, respectively. The lowest and the highest efficiency of the system occurred in the removal of nitrate and nitrite, respectively. Also, the adopted CWS promoted reasonable performance in the removal of pollutants (Tanner et al., 2005; Vymazal, 2007).

Constructed wetland systems remove pollutants through sedimentation, adsorption, accumulation of organic material, microbial assimilation, nitrification-denitrification, ammonia volatilization and removal by plants (Vymazal, 2011). Probably, for a higher adsorption to occur, it would be necessary for another type of filtering medium, instead of crushed stone, which has low capacity of retention for cations (Vymazal, 2014), or an increment in the hydraulic retention time (Matos et al., 2010).

N is an ion that is present in different forms in the effluent. N removal in CWSs starts after the transformation of organic N into inorganic N, a process called ammonification. Organic N can be converted into ammonia ($NH_3$) or ammonium ($NH_4^+$), and environments with pH close to or lower than neutrality lead to the predominance of $NH_4^+$. The ammonium ion can be absorbed by the crop and/or oxidized to nitrate through the process of nitrification (Saeed et al., 2012). Since the pH observed in the WDCF was close to or lower than neutrality, the ammonia may have been transformed into the ammonium ion (Nunes, 2012) and absorbed by the vetiver grass.

The observed removal of ammonia may also have occurred through the process of ammonia volatilization; however, this is not a desirable process, because a transfer of pollution would be occurring, since the $NH_3$ is an atmospheric pollutant and can contaminate aquatic and terrestrial environments, through dry deposition in soils or precipitation, reaching aquatic systems, causing acidification and eutrophication, and significantly altering natural ecosystems (Zhou et al., 2008).

There are different processes of removal of ammonia from the liquid fraction in CWSs, among which nitrification stands out, followed by denitrification. Nitrification is the biological process in which ammonia is oxidized to nitrite and the latter to nitrate, mostly by autotrophic bacteria called Nitrosomonas and Nitrobacter, respectively (Nunes, 2012). During the process of nitrification, nitrifying bacteria utilize the oxygen transferred from the atmosphere to the substrate through plant roots to oxidize the ammonia.

The nitrification process is highly influenced by environmental conditions; the pH and the presence of organic compounds, inorganic compounds and toxic substances are some of these influencing factors (Nunes, 2012). According to Nunes (2012), the pH considered as optimal for nitrification is between 7.2 and 8.0. The pH of the WDCF observed during the experimental period was, on average, 6.7, that is, below the optimal range for the occurrence of nitrification. This may have contributed to the occurrence of reduction in the ammonia removal efficiency. Additionally, the CWS used in the present study has horizontal subsurface flow, which shows anaerobic conditions compromising the process of nitrification because had no root system in the bottom of the CWS. Vymazal (2014) analyzed various studies conducted using CWSs and observed that, in one of the analyzed studies, the concentration of ammonia at the outlet of the CWS was superior to that at the inlet, which was attributed to the limited capacity of nitrification of the horizontal subsurface-flow CWS.

Therefore, ammonia may also be removed through anaerobic ammonia oxidation (ANAMOX), which occurs in environments with deficit of oxygen. Under these conditions, ammonium reacts with nitrite, generating molecular N ($N_2$) as the product (Saeed et al., 2012; Scheeren et al., 2011). This process plays an important role in constructed wetland systems where $NH_4^+$ and $NO_2^-$ coexist, thus stimulating the increment in N removal rates. It is also possible that part of the N has been immobilized and/or mineralized in the medium by microorganisms through biogeochemical pathways.

Matos et al. (2010), using constructed wetland systems in the treatment of swine farming wastewater (PFW), obtained total N removal efficiency of 64% in systems cultivated with Tifton 85, and 61% for systems cultivated with Alternanthera.

Ucker et al. (2012) obtained good efficiency in the removal of ammonia in CWSs cultivated with vetiver.

These authors observed removal efficiency values of 93 and 73% for CWSs cultivated with this species, varying the effluent depth level, and 42 and 43% in non-vegetated systems.

A higher efficiency of removal of N compounds by the CWS cultivated with vetiver grass, which has rapid and high capacity to remove nutrients such as N and P, besides large amounts of agrochemicals and heavy metals was expected (Ucker et al., 2012). Cheng et al. (2008) evaluated the performance of constructed wetland systems for an effluent that is disposed in one of the most polluted rivers of Taiwan, the Wu-Luo River, and obtained results of N removal from the effluent of about 93%.

According to Wu et al. (2014), traditional wetland systems constructed with horizontal subsurface flow often have low efficiency in the treatment of wastewaters, in comparison to other types of CWS, because the horizontal subsurface-flow CWS has a small amount of oxygen.

Despite the good removal of N, on average, for ammonia, TKN and nitrate, the effluent at the outlet of the CWS still had pollutant power. An important factor that may have promoted this result is the hydraulic retention time used in the present study, which was 33.6 h, because the lack of contact time necessary between the effluent and the substrate of the CWS can reduce its efficiency (Fia et al., 2010). Tanner et al. (2005) used cultivated beds in the treatment of wastewater from dairy cattle farming and obtained a total N removal of 79% in the first year and only 21% the second year.

According to Vymazal (2007), simple CWSs cannot achieve a high removal of total N, due to their inability to provide aerobic and anaerobic conditions, at the same time. This is because vertical-flow CWSs are able to successfully remove ammonia, but the denitrification is limited in these systems. On the other hand, according to the same author, horizontal-flow CWSs provide good conditions for denitrification, but the capacity of these systems for ammonia nitrification is very limited. Therefore, it is recommended to use more than one type of CWS to obtain better efficiency in the removal of this pollutant.

It should be highlighted that, along the present study, the CWS showed different values of efficiency, causing high amplitude in the removal of pollutants. In the samplings with the best results, it was observed that the treatment using horizontal subsurface-flow CWS was able to remove 76.5, 50.5, 37.4 and 64.4% of TKN, ammonia, nitrate and nitrite, respectively. These results are similar to those observed by many authors (Ucker et al., 2012; Matos et al., 2010; Tanner et al., 2005). It is important to point out that no chemical treatment was performed in the present study, only biological treatment for the removal of pollutants.

It was observed that the standard deviation of nitrate removal efficiency was superior to the mean of this parameter. This occurred because the nitrate concentration at the outlet of the CWS, in some samples, was superior to the nitrate concentration at the inlet; therefore, nitrate removal did not occur in some samples. Similar results were observed in the study conducted by Matos et al. (2010), who used CWSs in the treatment of swine farming wastewater. These authors suggest that the increase in nitrate concentration at the outlet of the CWS may have been due to the oxidation of ammoniacal forms, which probably caused the inefficiency of the system in the removal of nitrate.

The biological process that effectively removes the N from the treatment system is called denitrification. This reaction may occur in anoxic environments or environments with restricted oxygen, where nitrate will be reduced to nitrous oxide, which is released to the atmosphere. Nitrate can also be removed through absorption by the plant. Probably, in some samples, the vetiver grass does not absorb the nitrate present in the WDCF in an amount necessary for the occurrence of higher removal efficiency in the system, because its consumption by the plant is limited. The evapotranspiration of the system may also have contributed to this result (Ucker et al., 2012), besides the low retention of nitrate in the supporting medium.

According to Vymazal (2009), satisfactory results in wastewater treatment using CWS with horizontal subsurface flow have been reported by various authors. It should be pointed out that, although the CWS did not promote high efficiency in the removal of pollutants, it showed results similar to those observed by Stone et al. (2004) and Tanner et al. (2005). Therefore, the CWS with horizontal subsurface flow can be used in the post-treatment of wastewaters.

The vetiver grass used in the CWS showed good development in comparison to the data reported in the literature (Barbosa and Lima, 2013). The vetiver grass of average height was 1.4 m. Value above that observed by Andrade et al. (2011). The good performance of this crop, besides demonstrating its good adaptation to the CWS, shows that the WDCF probably provided the nutrients necessary to its development. In addition, no symptom of toxicity or deficiency of nutrients was observed in the vetiver grass. Ucker et al. (2012) used vertical- and horizontal-flow CWSs cultivated with vetiver grass and CWS without cultivation and observed higher N and P removal efficiency in CWSs cultivated with vetiver grass.

Studies have demonstrated that the CWSs with plants show higher efficiency of treatment, especially in the removal of organic matter and nutrients (Vymazal, 2011). According to Tee et al. (2009), this occurs due to the creation of microaerobic regions in the root zone of the plants in the CWS, which allows a faster rate of biodegradation and uptake of nutrients by plants.

It is emphasized that although the vetiver grass assist in the removal of pollutants, the plant can sometimes cause an increase in organic matter in the effluent due to the replacement of culture roots and thus increase the

concentration of pollutants. This can reduce system efficiency.

## Conclusions

Based on data observed in the literature, the constructed wetland system proposed cultivated with vetiver grass showed adequate efficiency in the removal of pollutants, with higher efficiency in the removal of nitrite, total Kjeldahl nitrogen and ammonia.

The vetiver grass exhibited good adaptation to the constructed wetland system, satisfactory development and there were no visual symptoms of nutrients deficiency.

For the same configuration of the constructed wetland system proposed in this work, it is recommended to use a larger hydraulic retention time for greater efficiency of the treatment system in removing pollutants.

## Conflict of Interests

The authors have not declared any conflict of interests.

## ACKNOWLEDGMENTS

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*Full Length Research Paper*

# Determination of wheat crop coefficient (Kc) and soil water evaporation (Ke) in Maringa, PR, Brazil

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**The objective of the present study was to determine the crop coefficient (Kc) of wheat and soil water evaporation (Ke) in Maringá, PR, Brazil. Wheat crop evapotranspiration (ETc) was determined through the water balance method, using drainage lysimeters integrated with soil moisture measurements. The ETo was calculated using the Penman-Monteith equation with climate data from an automatic weather station, then Kc was calculated. Microlysimeters were built, using PVC pipes with 100 mm diameter and 150 mm length, which were weighted every day to obtain the quantity of water evaporated and then the soil water evaporation coefficient (Ke) was calculated. The calculated values of Kc were compared with values presented by FAO 56. The calculated Kc were 0.67, 0.67, 1.01, 1.03 and 0.42 for tillering, stem extension, heading, flowering and ripening, respectively. The values of Kc presented high correlation and precision as compared to FAO model. The values of evaporation determined through microlysimeters were greater as compared to the ETo during the beginning of the experiment, when soil was uncovered and decreased during the crop development.**

**Key words:** FAO 56, wheat, drainage lysimeters, microlysimeters, evapotranspiration.

## INTRODUCTION

Accounting for only twenty percent of the world agriculture, the irrigated agriculture areas are responsible for 40% of world food supply. In contrast, irrigated agriculture consumes 80% of world's freshwater (Shiklomanov, 2000). The knowledge of water requirements by plants helps growers and researchers to improve the management of field activities, such as irrigation events. However, the irrigation water requirement is the total amount of water needed, in addition to precipitation, to satisfy the crop evapotranspiration (ETc) requirement.

The ETc is defined as the plant's transpiration and soil water evaporation systems under standard condition, where there is no stress by water quality constraints, pests or inadequate soil fertility (Allen et al. 2006). Souza and Gomes (2008) mentioned that the ETc can be determined by using water balance in the soil, which consists of monitoring of water storage, water output and

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water input in the soil during the certain period of time.

Different methods existent for determination of evapotranspiration, these methods are direct and indirect methods. The direct methods require the use of lysimeters and soil water balance to calculate the evapotranspiration during a determined period, while the indirect methods calculate the evapotranspiration using the theoretical or empirical equation with meteorological data (Cavalcante Jr et al., 2011). The widely used indirect method to determine the evapotranspiration is proposed by FAO, Food and Agriculture Organization of the United Nations, published in Bulletin No. 56 (Allen et al., 2006) due to the easy applicability and simplicity, in which only crop coefficient (Kc) values and meteorological data are needed.

The Kc is an empirical relation among a crop evapotranspiration (ETc), under ideal conditions, full development, and the evapotranspiration of a hypothetical reference crop (ETo). The Kc is intensely used to estimate crop water use and to schedule irrigation events. The FAO methodology was developed to provide growers with a simple ETc prediction tool for guiding irrigation management decisions. Values of Kc varies over the crop development and increases from a minimum value at planting until a maximum Kc reaches full canopy cover, the Kc tends to decline at a point after a full cover is reached in the crop season (Allen et al., 2006; Ko et al., 2009).

Several studies determine the values of Kc for different locations. Libardi and Costa (1997) studied wheat Kc for Piracicaba, midwest of Brazil, and Kc values ranged from 0.33 at tillering to 1.16 at flowering. In addition, Kc values were 0.79 at stem extension, 1.11 at heading and 0.45 at ripening. In another study developed in the Cerrado region of Brazil, values of Kc were 0.99 at tillering, 1.12 at stem extension, 1.43 at heading and flowering and decreased to 0.45 at ripening, the phenological stages duration were 22, 18, 48 and 12 days, respectively (Guerra and Jacomazzi, 2001).

The comparison between local Kc and the existent FAO values is always performed to ensure the quality of the new values (Araujo et al., 2011; Cavalcante Jr et al., 2011; Rácz et al., 2013; Filho et al., 2015; Kisi, 2016). A study developed in Texas (USA) found coefficient of Pearson of 0.87 for the new values of Kc for wheat as compared to the FAO value (Ko et al., 2009). Cavalcante Jr. et al. (2011) determined the coefficient of sunflower crop and obtained values of 0.87 and 0.76 for coefficiency of Pearson, and Willmott index respectively, when compared with the values proposed by Allen et al. (2006). Furthermore, there are few other data analysis methods available as statistical comparison index, such as root mean square error (RMSE) and coefficient of determination ($R^2$).

The soil water evaporation coefficient (Ke) has high influence in determining the Kc during the initial phase of crop development when the soil is most exposed and the crop transpiration capacity is reduced. The evaporation component varies daily according to surface soil moisture. The transpiration component has more stable behavior, being tabulated in ranges of variation for each phase of crop development (Allen et al., 2006).

The rate of soil water evaporation can be grouped into different stages. The first stage can comprise from one to three days, depending on the magnitude of the evaporation rate of this stage, which depends on the atmospheric conditions and is approximately 90% of the evaporative requirement. The length of this stage is influenced by the rate of evaporation, soil depth and soil hydraulic properties (Freitas et al., 2006). The second stage occurs when soil surface becomes drier and evaporation occur beneath the surface. The water vapor reaches the surface by molecular diffusion and mass flow, caused by fluctuating of air pressure.

Studies using direct measurement methods to determine evapotranspiration have been performing to determine the soil water evaporation. The use of lysimeter properly installed performs precise evaporation measurements for the different layers set up (Carvalho et al., 2007). Furthermore, microlysimeters is an option to measure evaporation of discovered and cultivated soil (Burt et al., 2005). The microlysimeter is PVC pipe filled up with soil, installed between crop rows and weighted periodically to calculate the mass difference by evaporation during the period desired. Flumignam et al. (2012) compared the water evaporation from the soil lysimeters and microlysimeters, and concluded that the use of microlysimeters is valid. Dalmago et al. (2010) validated the use of microlysimeters for absolute measurements and relative values of water evaporation from the soil to compare the planting methods.

Although, the FAO methodology is a great tool to determine the crop water requirement, there are weather variabilities over different locations, as well as values of Ke and Kc for all crop development stages. Standard values should be used only in the absence of local values; therefore, the objective of the study was to determine the crop coefficient (Kc) of wheat and soil water evaporation (Ke) and to compare the values with the methodology proposed by the FAO No. 56 bulletin.

## MATERIALS AND METHODS

The study was conducted at the experimental field at the Irrigation Training Center of State University of Maringá, located in Maringá, PR, Brazil (Latitude 53°25', Longitude 51°56' and Altitude 542 m). The soil at the research area is classified as Eutroferric Red Latosol, according to EMBRAPA (2006), typically found in Maringa. The soil has 8% of the slope with a clay texture, the proportion of particle fractions are 6% sand, 13% silt and 81% clay. The climate of Maringá is Cfa - Humid Subtropical Climate, according to the classification of Koppen. The rainy period occurs from December to February, characterized as summer, while from July to August are the dryer months, winter.

Three drainage lysimeters were installed in the experimental area, with a volume of 2 $m^3$, the soil was set inside each lysimeter

**Table 1.** Performance of the comparison coefficient (indicator "c") developed by Camargo and Sentelhas (1997).

| "c" Indicator values | Performance |
|---|---|
| >0.85 | Great |
| 0.76 a 0.85 | Very good |
| 0.66 a 0.75 | Good |
| 0.61 a 0.65 | Medium |
| 0.51 a 0.60 | Poorly |
| 0.41 a 0.50 | Very poorly |
| ≤ 0.40 | Extremelly poorly |

Source: Camargo and Sentelhas (1997).

to mimic soil layers from the soil profile around the lysimeters. In addition, in the bottom of each lysimeter, a layer of stone gravel and sandy covered by a grid were set up to avoid soil leaching. Soil moisture was controlled using time domain reflectometry (TDR) sensors (TRASE 6050X1, Soil Moisture Equipment Corp). TDR sensors were installed in each lysimeter at 5, 15, 25, 35 and 45 cm of soil depth and data was collected every day.

The water balance from irrigation, precipitation, drainage and soil moisture variation was used to calculate the crop evapotranspiration (ETc). The non-uniform water movement into the soil resulted in a greater water drainage close to the water supply than farther areas; however, water drainage reduced over time. The soil water balance was calculated in timing intervals between rainfall events or irrigation events, using the following equation 1:

$$ETc = \frac{\Delta A\ (\Sigma P + \Sigma I + \Sigma D)}{N_p} \quad (1)$$

Where: ETc- crop evapotranspiration, calculated by data collected from lysimeters (mm $d^{-1}$); ΔA- storage variation in a specific time, data from TDR (mm); P- precipitation between events (mm); I- irrigation, total of water applied between events (mm); D- total of water drainage between events (mm); Np- days between events.

To calculate the reference evapotranspiration, data were collected from the automatic weather station installed in the field experiment, and used in the Equation 2. The Equation 2 was estimated by Penman-Montheith method, as recommended by FAO No. 56 bulletin (Allen et al., 2006).

$$ETo = \frac{0.408\Delta.R_n - G) + \gamma \frac{900}{T+273} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0{,}34 u_2)} \quad (2)$$

Where: ETo- daily evapotranspiration reference (mm $d^{-1}$); Δ- slope the vapor pressure curve at the point T (kPa $ºC^{-1}$); γ- psychrometric constant (kPa $ºC^{-1}$); Rn- daily solar radiation balance (MJ $m^{-2}$ $d^{-1}$); G- daily flow of soil heat (MJ $m^{-2}$ $d^{-1}$); λ- latent heat of vaporization (MJ $kg^{-1}$); T- average daily air temperature (ºC); $U_2$- average daily wind speed from 2 m height; $e_{s-}$ average daily saturation pressure of water vapor (kPa); $e_a$- average daily pressure of water vapor (kPa).

The Kc (Equation 3) was determined by the ratio of the ETc average values, which was obtained during events between two points, or the ETc average in this same period, the result represents the Kc in number of days, which were placed within each phase of the culture seeking to obtain the medium value of Kc of each phenological stage proposed by Allen et al. (2006).

$$Kc = \frac{ETc}{ETo} \quad (3)$$

To determine Ke, four PVC microlysimeters, 100 mm diameter and 150 mm height with the bottom sealed by a lid, were installed between the wheat rows. During all the seasons, the micro-lysimeters were daily weighted and the difference in mass between days determined to evaluate the daily evaporated water.

To quantify the amount of water in the mycrolysimeter, a baker was used, which allowed supplying the same quantity of water to lysimeters. The mean values from microlysimeter were used to quantify the evaporation values or Ke, according to the Equation 4.

$$Ke = \frac{Evaporation}{ETo} \quad (4)$$

Where: Evaporation- evaporation from microlysimeters (mm).

The methodology for calculating the Kc and Ke from FAO-56 is described in Allen et al. (2006), where the tabulated values are corrected by the local climatological data, and the Kc and Ke for each region were estimated.

The comparative analysis between the estimated and determined ETc was performed by a statistical comparison index, suggested by Camargo & Sentelhas (1997). The degree of accuracy was obtained by the correlation coefficient of Pearson "r" (Equation 5), the accuracy was evaluated by Willmott index "d" (Equation 6) and the performance by the indicator "c". Where "c" obtained by the product of the ratio "d" and coefficient "r". The values of indicator "c" classified according to Table 1.

$$r = \frac{\sum_{i=1}^{N}(O_i - O)*(P_i - P)}{\sqrt{\sum_{i=1}^{N}(O_i - O)^2} * \sqrt{\sum_{i=1}^{N}(P_i - P)^2}} \quad (5)$$

$$d = 1 - \left[ \frac{\sum_{i=1}^{N}(P_i - O_i)^2}{\sum_{i=1}^{N}\left(|P_i - O| + |O_i - O|\right)^2} \right] \quad (6)$$

Where: Pi- estimated value; P- average of the estimated value; Oi- determined value; O- average of the determined values. Considering that for the coefficient "r"- 0.70 up or down indicates a strong correlation; 0.30 to 0.70 positive or negative indicates a moderate correlation; 0 to 0.30 indicates a weak correlation. For the index "d"- values range from zero (for no concordance) to 1 (for total concordance).

## RESULTS

The wheat development stages were classified in tillering, stem extension, heading, flowering and ripening (Large, 1954). The growth stage duration was 25, 22, 17, 15 and 20 days for tillering, heading, flowering and ripening, respectively. The life cycle of wheat was 99 days.

The cumulative rainfall during all crop development was 316.6 mm, and the total irrigation applied was 292.9 mm. The total water drainage during all field trial was 254 mm and the drainage was directly related to rainfall events. In particular, at 20 and 21/06, the water drainage had its

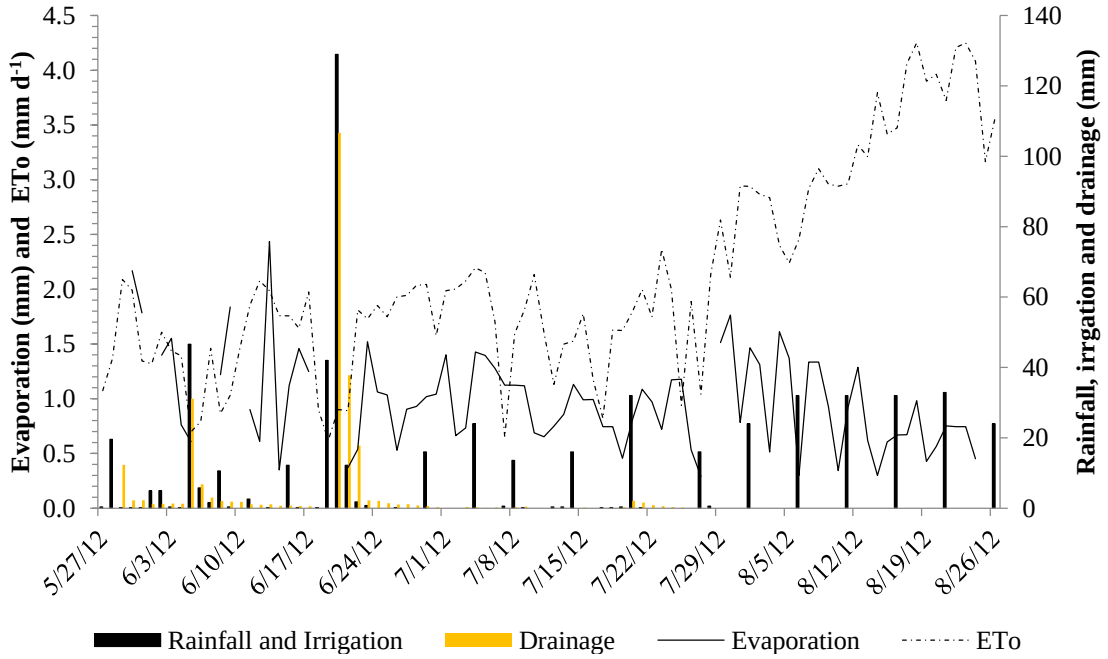


**Figure 1.** Cumulative rainfall and irrigated water, water drainage, ETo and soil water evaporation determined through microlysimeters during the experimental field.

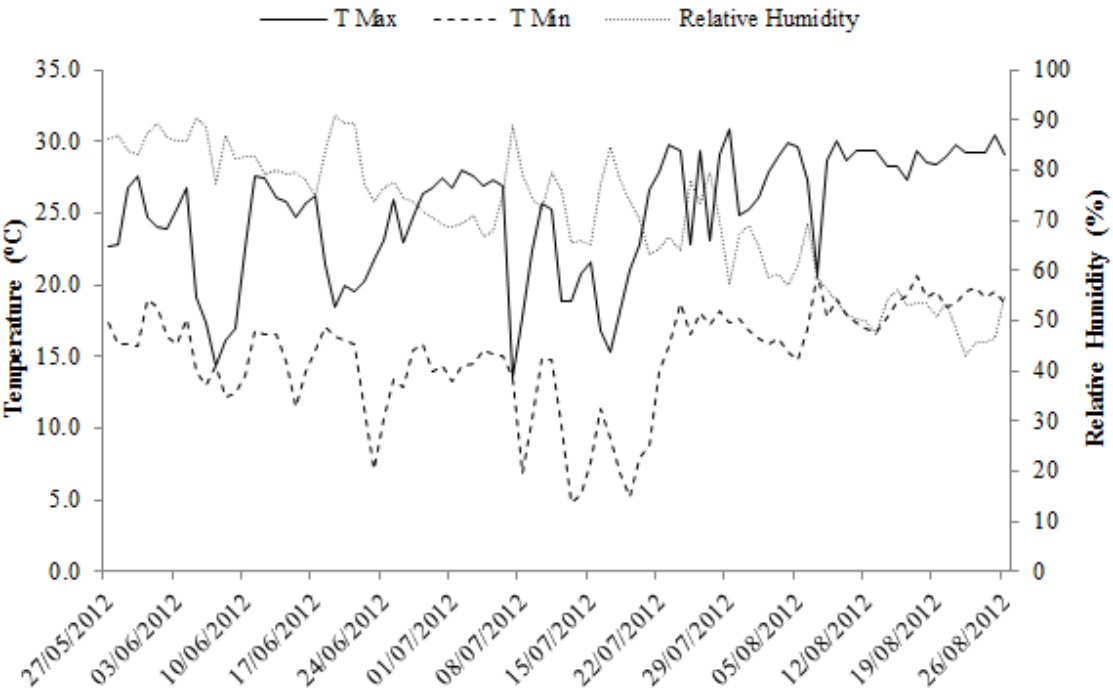


**Figure 2.** Maximum and minimum temperature and relative humidity during the field experiment.

maximum value (144 mm), in response to a rainfall event of 183 mm at 19, 20 and 21/06 (Figure 1).

The average temperature for the crop season was 19.5°C, with maximum and minimum temperature of 24.6 and 11.8°C, respectively. The relative humidity average was 70.8% (Figure 2). The daily solar radiation and wind speed presented in Figure 3 were used to calculate the ETc. Weather variables were directly affected by rainfall

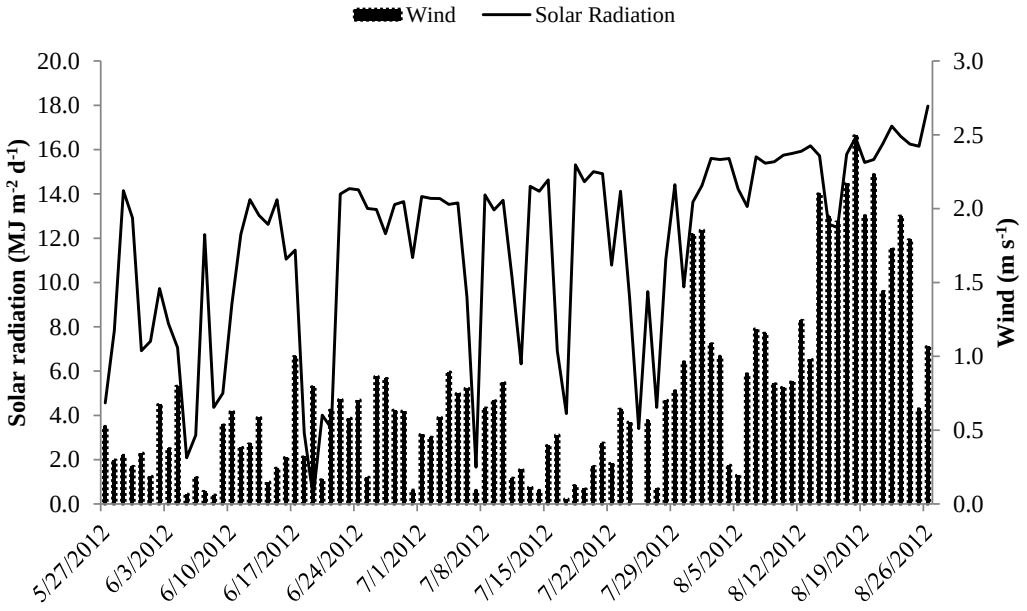


**Figure 3.** Solar radiation and wind speed during the field experiment.

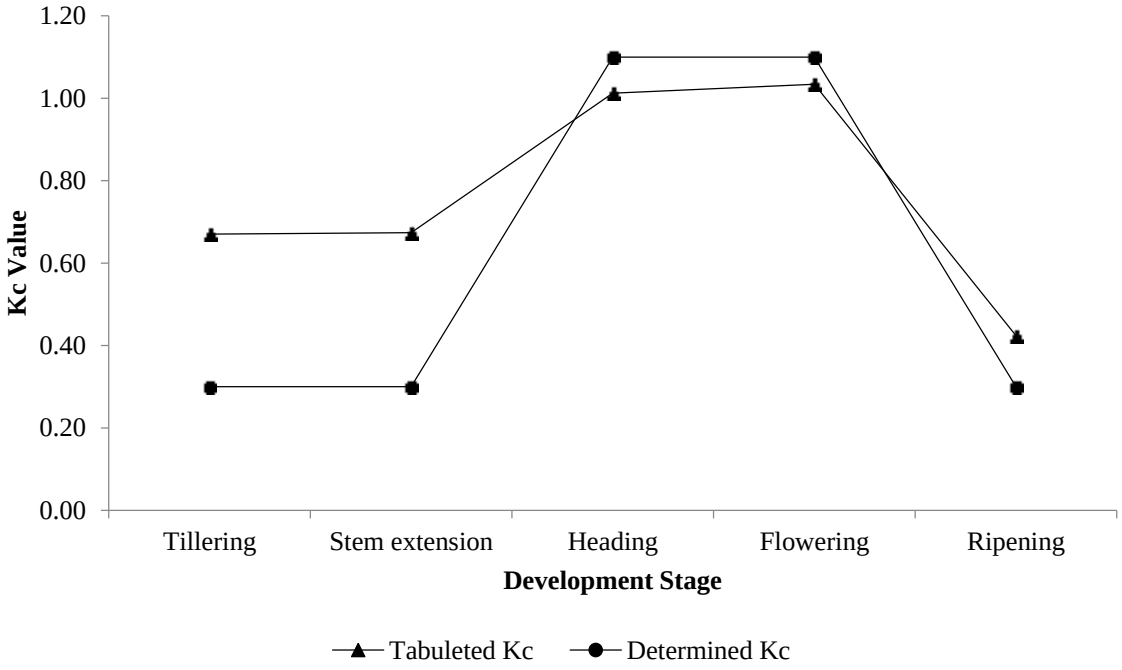


**Figure 4.** Comparison between the study determined values of Kc and the FAO-56 tabuleted Kc values for each wheat development stages (tillering, stem extension, heading, flowering and ripening).

events, in the presence of rain there is a decrease in daily solar radiation, which can be observed at the end of the crop season. The average solar radiation was 11.8 MJ $m^{-2}$ $d^{-1}$ and average wind speed was 0.7 m $s^{-1}$.

The calculated Kc values were 0.67, 0.67, 1.01, 1.03 and 0.42 for tillering, stem extension, heading, flowering and ripening growth stages (Figure 4), respectively. The Kc values were compared with values from FAO-56 and the coefficients were 0.952 for Pearson, 0.946 for Willmott index and 0.9 for the indicator 'c' from Camargo

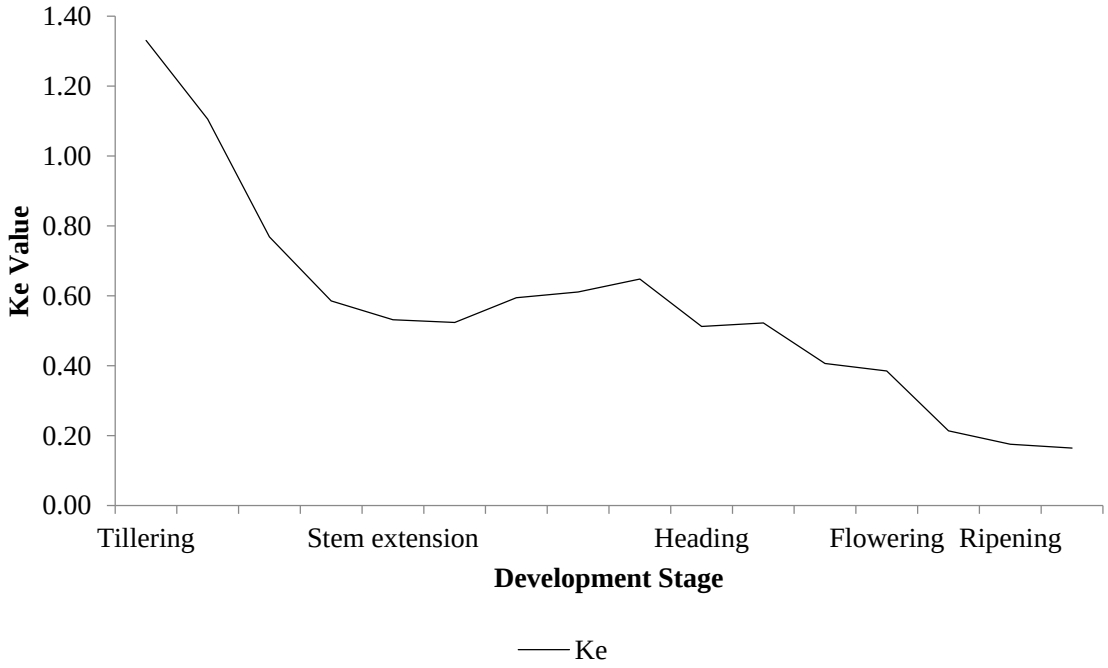


**Figure 5.** Controlled values of Ke during the wheat experimental development stages (tillering, stem extension, heading, flowering and ripening).

**Table 2.** Values of field determined Ke and estimated Ke values using the FAO-56 methodology for each wheat growth stages (tillering, stem extension, heading, flowering and ripening).

| Development stages | Ke | |
|---|---|---|
| | Determined | Estimated FAO |
| Tillering | 1.090 | 0.900 |
| | 1.092 | 0.869 |
| | 0.945 | 0.900 |
| | 0.589 | 0.900 |
| Stem extension | 0.499 | 0.500 |
| | 0.530 | 0.500 |
| | 0.600 | 0.500 |
| | 0.741 | 0.500 |
| Heading | 0.680 | 0.192 |
| | 0.568 | 0.179 |
| | 0.493 | 0.172 |
| Flowering | 0.415 | 0.130 |
| | 0.406 | 0.138 |
| | 0.117 | 0.105 |
| Ripening | 0.177 | 0.900 |
| | 0.142 | 0.900 |

and Sentelhas (1997).

The Ke was calculated over the season according to the FAO-56 methodology and expressed in Figure 5. Despite the ripening growth stage, the determined Ke values for all wheat growth stages were similar to those estimated by FAO-56 (Table 2). All Ke values were analyzed with those estimated by FAO-56 and the comparison coefficients were 0.348 for Pearson, 0.628

**Table 3.** Comparision coefficient for the study determining Ke values and estimated by FAO 56 methodology.

| Coefficient of Pearson "r" | Willmott Index "d" | Indicator "c" from Camargo and Sentelhas |
|---|---|---|
| 0.348 | 0.628 | 0.219 |

**Table 4.** Comparision coefficient for the study determining Ke values and estimated by FAO 56 methodology, excluding the ripening stage.

| Coefficient of Pearson "r" | Willmott Index "d" | Indicator "c" from Camargo & Sentelhas |
|---|---|---|
| 0.779 | 0.790 | 0.615 |

for Willmott index and 0.219 for the indicator ‘c’ from Camargo and Sentelhas (1997) (Table 3). However, when ripening Ke values are not included in the analysis with the Ke values of other wheat growth stages, there is an increase in comparison coefficients. The Pearson coefficient was 0.779, Willmott index was 0.790 and 0.615 for the indicator ‘c’ from Camargo and Sentelhas (1997) (Table 4).

## DISCUSSION

The wheat has a lot of development under temperatures of 18 and 24°C; however, each growth stage has an optimum temperature of 11.7, 23.0 and 21.3°C for stem extension, flowering and ripening, respectively (Farooq et al., 2011). The maximum and minimum temperatures increased over the crop development, which matches with the beginning of winter. The higher temperatures at plants senescence reduced the relative humidity, and both were influenced by the low precipitation presented during the growth stage, as compared to early season. The wind was used to calculate the ETo and had no direct effect on crop development, under moderated wind conditions; the crop canopy has the above ground moist air layer replaced by a typically drier air, which provides a high vapor pressure gradient that increases the evapotranspiration. The study determined Kc and FAO-56 values had similar trend over the wheat crop development (Figure 4), and the comparison coefficients had acceptable values, which were similar to those measured by Liu and Luo (2010), Cavalcante Jr et al. (2011), Neto et al. (2011) and Cavalcante Jr et al. (2013).

The differences between calculated and FAO-56 Kc values indicates the necessity of FAO-56 values adjustments for each wheat growing location. During initial growth stages, the determined Kc was different from the FAO-56 of 0.37 for tillering and stem extension, respectively. However, this difference was reduced with the crop development. At tillering and stem extension, most of the soil is bared, which increase soil water evaporation and explain such difference of both growth stages. Once the crop canopy is completely covering the soil, the influence of water evaporation on determining the evapotranspiration is reduced because of a reduction in soil water evaporation (Allen et al., 2006) and the determined values were closer to FAO-56.

The microlysimeters were a useful tool to estimate the soil water evaporation, although the equipment must be careful managed during the data collection and data analysis. The same was stated by Dalmago et al. (2010) and Flumignam et al. (2012). The Ke values had high comparison coefficients when ripening growth stage data were not included in the analysis.

The ripening Kc value was removed from the data analysis because at the end of the crop development when plants maturation process is ending, water was no longer required and irrigation was cut off. Microlysimeters received no water anymore and even with a reduction in leaf area due to crop maturation, which usually increases Ke values, the amount of available water for evaporation within microlysimeters was very small.

Ke responses to canopy closing are inversely proportional to Kc, when soil is bared, which usually occurs in the initial stages of crop development, the values of Ke is extremely high. The high Ke is explained by the high soil water evaporation; however, as leaf area increases the soil was covered and water evaporation reduced, consequently Ke was also reduced. The Ke plateau occurred when the soil was completely covered by crop canopy.

During the crop development, the soil water evaporation was quietly similar to ETo at initial crop growth stages, when transpiration was low or zero. The crop transpiration increased as crop advanced for next growth stages, there was an increasing in soil water uptake and decreasing in soil available water for evaporation. In addition, the proportion of covered soil by plant leaf area was increased as plants were under development, making difficult the incidence of solar radiation on the soil and reducing soil water evaporation. At plant senescence, the leaf area and transpiration rate were reduced and soil evaporation should increase; however, rainfall events decreased and irrigation ceased in the late season;

consequently, soil water evaporation reduced.

## Conclusion

The study concluded that the FAO-56 values of Kc for each crop development stage of wheat have to be determined for the desired growing region, to fix local weather conditions. The wheat crop coefficients for the studied region were 0.67 for the tillering stage; 0.67 for the booting stage; 1.01 during the flowering stage; 1.03 during grain filling; and 0.42 for the maturation stage to Maringá, PR, Brazil. These values can be used for similar weather conditions and will help researchers and growers to determine the wheat water requirements over the season.

The soil water evaporation during wheat development induced high initial values of ETo when the crop canopy had not fully covered the soil. The well developed crop reduced soil water evaporation. At the end of the cycle, another decrease was observed, because there was no water supply and increased evaporative demand is required by ETo.

When grain filling data were used and the system water supply was constant, the Ke values obtained by using the microlysimeters showed high correlation but low accuracy with the methodology proposed by the 56 FAO bulletin.

## Conflict of Interests

The authors have not declared any conflict of interests.

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