# African Journal of Agricultural Research

Volume 10 Number 28 9 July 2015

ISSN 1991-637X

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African Journal of Agricultural Research

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academicJournals

Vol. 10(28), pp. 2668-2679, 9 July, 2015
DOI: 10.5897/AJAR2015.9896
Article Number: 6EAC94B53952
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

*Full Length Research Paper*

# Study of ergonomical characteristics of farm workers

**Sirisha Adamala**

Research Scholar, Land and Water Resources Engineering, IIT Kharagpur, India.

Received 9 May, 2015; Accepted 18 June, 2015

**Ergonomics is system design compressed of man-machine and environment relationship to bring about some output against some given inputs considering different physical, anatomical, physiological, and psychological limits of human being. Based on different workloads (N) and speed (rpm), the experiment was conducted on MONARK 828E Ergometer, to evaluate heart rates of different age groups with variable weights and heights. The farm workers (both men and women) of 20-30 years age group have developed a maximum working heart rate as compared to other age groups of 31-40 and 41-50 years. The energy expenditure rate (EER) for all the farm workers obtained were graded as 'moderately heavy'. The body mass index (BMI) of age groups 20-30 and 31-40 years were considered as 'Normal' and 41-50 were considered as 'Obese Grade I'. The ponderal index (PI) of all age groups, that is, 20-30, 31-40, and 41-50 years were 'Ectomorph' in nature. Further, statistical analysis was performed to develop regression based prediction equation for EER using age, weight, height, heart rate, and maximal oxygen uptake (VO2max) as input variables.**

**Key word:** Ergonomics, heart rate, energy expenditure, ponderal index, body mass index.

## INTRODUCTION

Ergonomics is the scientific study of the relationship between man and his working environment. It is concerned with ways of designing machines, operations, and work environments so that they match human capabilities and limitations. Its goal includes increasing the productivity in operations, decreasing the amount of effort needed to operate machines, increasing human comfort during work, decreasing accidents, and eliminating error provocative features. In brief, it can be said that the application of ergonomics aims at heightening the quality of life in work conditions. The productivity of any work environment not only depends on the quality and complexity of the tools being used, but also on the human being involved towards operating the tool and his work environment (Agrawal and Satapathy, 2006).

Normally while designing the machine human take almost care on the technical part of the machine, but they do forget about the operator. As a result, most of the controls and displays are positioned beyond normal human accessibility or they have to be operated in very cramped awkward posture. For any ergonomist, it is therefore essential to design the work environment based on the anthropometric limitations of the operations. The human body is built for action not for the rest. Once upon a time this was a necessity: the struggle for survival

E-mail: sirisha@agfe.iitkgp.ernet.in

demanded good physical condition. But the optimal function can only be achieved by regularly exposing the heart, circulation, muscles, tendons, skeleton, and nervous system to some loading and training. In olden days, the body got its exercise both at work and at leisure. In modern society, however, machines have taken over an ever increasing share of the tasks which are formerly accomplished with muscular power alone. Many operations increase the stress on the labor and machine operators. This stress results in an increase of heart rate (HR), energy expenditure rate (EER), and decrease of maximal oxygen uptake ($VO_{2max}$) rates. The sensitivity of above three parameters depends mainly on age, weight, and height of farm workers.

In most form workers (men and women), HR monitoring provides one of the most efficient and economical means of estimating EER. In a range of approximately 90-150 bpm, which is of "flex heart rate", the relationship between HR and $VO_{2max}$ is linear (Rennie et al., 2001). Instead of linear relationship, a non-linear, discontinuous function is found to be more accurate in predicting EER from HR and $VO_{2max}$. In addition to HR and $VO_{2max}$, the factors age, weight, and height may have a significant effect in EER prediction.

Most of the previous studies have used individual calibration of the HR and EER in the prediction of energy expenditure (Luke et al., 1997). This requires that each farm worker should complete a progressive exercise test, during which HR is simultaneously measured, along with indirect calorimetry to estimate EER. This procedure is very time consuming and requires well trained healthy and active human power to complete the test. These limitations demonstrated the utility of developing prediction equations for estimating EER from the HR, age, weight, height, and $VO_{2max}$ in representative samples of individuals, with reasonable accuracy and the potential for wide application in epidemiological studies.

Hiilloskorpi et al. (1999) developed a multiple linear (MLR) regression based prediction equation for EER from HR on a sample of 87 healthy, active subjects (men and women). Results found that the age, weight, and gender had a significant interaction with EER. Similarly, Rennie et al. (2001) developed a MLR based prediction model using a sample of 789 individuals for estimating EER from sitting HR, age, weight, and gender. The EER prediction equation was then further validated on an independent sample of 97 individuals and found to have a correlation coefficient (r) of 0.73.

Therefore, the present study is carried out with the following objectives:

1. To study the ergonomical characteristics of farm workers such as HR, $VO_{2max}$, EER, Body Mass Index (BMI), and Ponderal Index (PI).
a. With different age groups of male farm workers (20-30, 31-40, 41-50 years).
b. With different workloads (N) and speed (rpm).
c. With different heights (cm) and weights (kg) of workers.
2. To develop a prediction equation for EER from age, weight, height, HR, and $VO_{2max}$ as input variables.

## MATERIALS AND METHODS

### Monark Ergometer cycle 828E

This experimental analysis was done at Instrumentation Lab in the campus of College of Agricultural Engineering, Bapatla, Andhra Pradesh (India) using Monark cycle Ergometer 828E (Figure 1). The Monark Ergometer model 828E is a completely housed flywheel and further strengthened the frame. It is specially made for efficient exercise where one get immediate feedback and it has an adjustable brake system, where the brake can be set/read in Newton (N). The dependent variables are age, weights, and heights, time, workloads (N), speed (rpm), HR (beats per minute, bpm), and $VO_{2max}$ (ml/kg/min), whereas the EER(kJ/min) is independent variable. The farm workers three in each age group of (20-30, 31-40, and 41-50) free from respiratory or any serious health problem were selected for the study (Table 1). A total of 39 farm workers are volunteered to participate in the study. The farm workers (men and women) represented a wide range of morphology and fitness: age 20–50 years of age, body weight 42–92 kg, heights 152-185 cm, HR 172-195 bpm, $VO_{2max}$ 59.5-72.7 ml/kg/min, and EER 44-53 kJ/min.

The univariate (means and standard deviations) statistical summary of farm workers is shown in Table 2. There were no differences in mean age and $VO_{2max}$ between the men and women participants who underwent cycling using Ergometer. There were significant differences in weight, height, maximum working heart rate, and EER between the sexes (Table 2, $P<0.05$). Based on this analysis, a mixed model for predicting EER is fitted in following sections. The factors age, weight, height, HR, and $VO_{2max}$ were modeled as fixed effects to predict EER.

The cycle is equipped with an electronic meter (Figure 2) showing pedal revolutions per minute (rpm), heart rate (bpm), exercise time (min), an imagined cycling speed (kph), covered the distance (km), burned calories (cal), and the power on the cycle (W).

The product includes heart tronic's new integrated transmitter/belt. This device is water resistant and may used for all water related exercises. Attach the elastic strap to the transmitter (Figure 3). Observe the each end of the transmitter has an open slot and two teeth extended slightly from the end. The heart tronic logo should be centered on the chest.

Switch ON the instrument by pressing the Power ON switch. Initially, the beat LED will flash erratically. After a few seconds, it will stabilize and will flash in accordance with the heart beat. With every LED flash a buzzer (beep) sound will be heard (Figure 4). It is very important to fix the electrodes in the proper way. Wash the skin and remove hair on the site where the electrodes are going to be placed. There should be no cut marks on the skin. Remove the transparent plastic cover sheet. Place the electrodes on the three sites (Figure 5). Then clip the sensor lead buttons to the electrodes. The weighing balance and metal wire tape has been used for measuring the weights and heights of the subjects.

### Protocol of ergonomical tests

The Monark 828E software is an easy-to-use package, designed to work with Monark 828E ergometer. By using this software, three


**Figure 1.** Monark Ergomedic cycle 828E.


**Figure 2.** Electronic meter.


**Figure 3.** Heart rate monitor transmitter belt.


**Figure 4.** Polar heart rate monitor.

tests Astrand, WHO, and YMCA are performed.

### *Astrand test*

The 'Astrand protocol' is designed to determine the $VO_{2max}$ by exercising the farm workers on Ergometer at a sub maximal workload and measuring the steady state HR. The workload, in conjunction with the resultant heart rate, is compared to the predicted relationship, adjusted for age and sex and $VO_{2max}$ is computed.

### *WHO test*

This protocol is recommended by World Health Organization (WHO), because it is considered reliable and accurate, and it is in widespread use in many health clubs and physiological research centers throughout the world. The test uses branching multiple workloads, and takes into account the executor's age, weight, and sex. This means that the workload is adjusted at each level to correspond those values that the WHO has recommended.

### *YMCA test*

The YMCA protocol is based on the "Y's Way to Physical Fitness" bicycle test. The design is a sub maximal test; using branching multiple workloads in which the next work load is determined by the steady state heart rate elicited by the previous level. Every three minutes the work load is advanced until the farm worker has completed three levels requiring a total of nine minutes. If the subject has an abnormally high heart rate response to the initial work load (110 bpm or more), the test is terminated at the end of

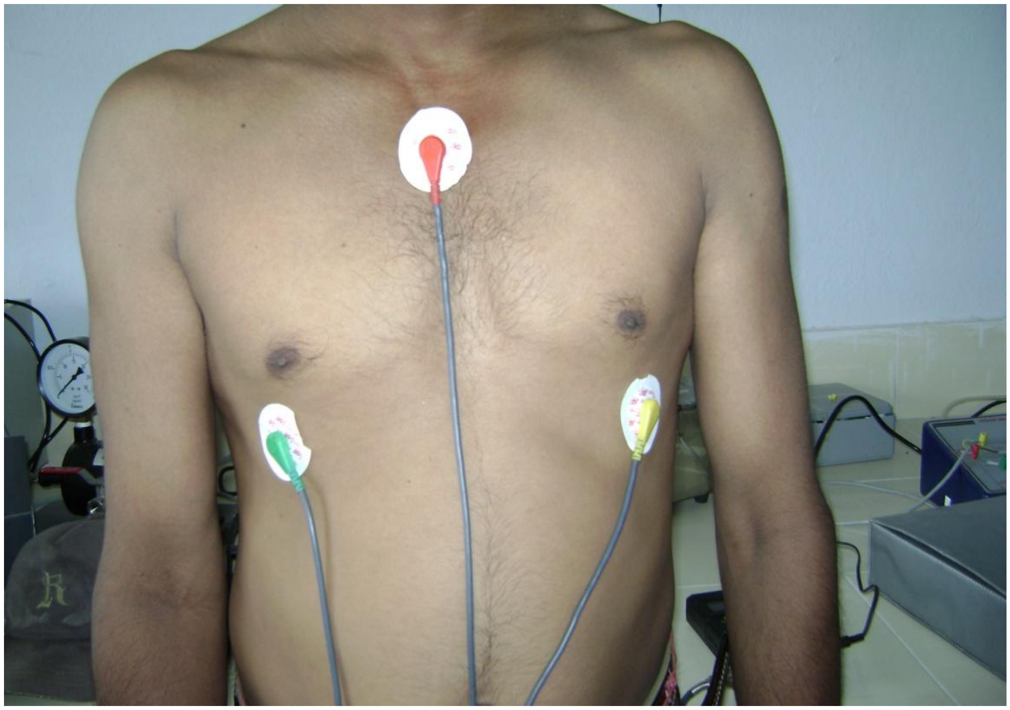

**Figure 5.** Arrangement of electrode leads (green, red and yellow buttons) to the farm worker for measuring the heart rate at rest position.

second level. If the operator senses that the subject is experiencing difficulty completing the third level of the protocol, the test can be concluded before the third level is finished. If the test is aborted prior the end of the second level, no $VO_{2max}$ calculation can be done.

### Computation of parameters

#### *Energy expenditure rate (EER)*

The EER is calculated using the following empirical relationship:

$$EER = (HR-66)/2.4 \quad (1)$$

Where EER = Energy Expenditure Rate (kJ/min) and HR = heart rate (beats/min).

#### *Body mass index (BMI)*

BMI of the selected subjects was calculated by using the following formula:

$$\text{BMI} = \frac{(\text{Weight, kg})}{\text{Height}^2\ (\text{m})} \quad (2)$$

#### *Ponderal index (PI)*

Body composition was assessed using PI as given below:

$$\text{PI} = \frac{(\text{Weight, kg})}{\text{Height}^3\ (\text{m})} \quad (3)$$

## RESULTS AND DISCUSSION

### Variation of HR of farm workers with different workloads (9.8, 14.7, and 19.6 N) at 50 rpm

The variation of HR of farm workers during cycling Ergometer with different workloads (9.8, 14.7, and 19.6 N) at 50 rpm speed is shown in Table 3. At a workload of 9.8 N, the mean values of increased working heart rate (1-6 min) and decreased resting heart rate were 126.17 and 98.3 bpm graded as ‘heavy’ and ‘light’, respectively (Zander, 1973). The EER varies from a minimum of 21.67 kJ $min^{-1}$ to a maximum of 28.75 kJ $min^{-1}$. The mean of EER was 25.07 kJ $min^{-1}$ and it is graded as moderately heavy’ (Zander, 1973).

At 14.7 N workload, the mean values of increased working heart rate (1-6 min) and decreased rest heart rate are 136.17 and 107.3 bpm, respectively graded as ‘heavy’ and ‘moderately heavy’ (Zander, 1973). The EER varies from a minimum of 24.58 kJ $min^{-1}$ to a maximum of 33.75 kJ $min^{-1}$. The mean of EER was 29.235 kJ $min^{-1}$ and it is graded as ‘moderately heavy’ (Zander, 1973).

At 19.6 N workload, the mean values of increased

**Table 1.** Details of the farm workers involved in the pedaling or cycling of Ergometer.

| Age group (years) | Name of the operator | Sex | Age (years) | Weight (kg) | Height (cm) | Maximum working HR (bpm) | $VO_{2max}$ (ml $kg^{-1}$ $min^{-1}$) | EER (kJ/min) |
|---|---|---|---|---|---|---|---|---|
| | N. Ashok Kumar | Male | 27 | 62 | 170.2 | 192 | 70.82 | 52.52 |
| | N. Surya Prakash | Male | 25 | 68 | 170.2 | 194 | 70.26 | 53.33 |
| | S. Venkat Reddy | Male | 26 | 52 | 157.5 | 193 | 72.74 | 52.91 |
| | K. Tirupati | Male | 22 | 70 | 168.5 | 190 | 71.71 | 51.66 |
| | R. Prasad | Male | 29 | 59 | 163.0 | 189 | 71.02 | 51.25 |
| | K. Sathyanarayana | Male | 27 | 72 | 179.0 | 192 | 69.12 | 52.50 |
| 20-30 | V. Shankar | Male | 26 | 80 | 165.0 | 185 | 69.25 | 49.58 |
| | B. Bhuchanna | Male | 22 | 92 | 172.0 | 186 | 68.61 | 50.00 |
| | D. Rajayya | Male | 30 | 77 | 185.0 | 195 | 66.64 | 53.75 |
| | A. Narsakka | Female | 20 | 45 | 155.0 | 185 | 72.19 | 49.58 |
| | Ch. Manga | Female | 21 | 48 | 157.0 | 189 | 70.67 | 51.25 |
| | N. Bhulaxmi | Female | 25 | 42 | 152.0 | 183 | 71.08 | 48.75 |
| | G. Vani | Female | 30 | 44 | 156.0 | 182 | 68.96 | 48.33 |
| | N. Thirupathayya | Male | 33 | 56 | 157.5 | 189 | 69.98 | 51.25 |
| | S. Srinivasa Rao | Male | 34 | 62 | 157.5 | 188 | 68.73 | 50.83 |
| | K. Srinu | Male | 34 | 74 | 167.6 | 188 | 66.69 | 50.83 |
| | Ch. Kutumba Rao | Male | 39 | 88 | 182.3 | 190 | 62.07 | 51.67 |
| | M. Mallikarjuna | Male | 32 | 90 | 176.2 | 189 | 64.60 | 51.25 |
| | N. Veeranna | Male | 36 | 76 | 165.2 | 172 | 68.08 | 44.16 |
| 31-40 | U. Gangaram | Male | 38 | 63 | 181.2 | 185 | 67.48 | 49.58 |
| | A. Daniel | Male | 33 | 75 | 175.0 | 188 | 66.91 | 50.83 |
| | U. Sukumar | Male | 32 | 70 | 168.0 | 187 | 68.30 | 50.41 |
| | M. Padmaja | Female | 30 | 60 | 155.0 | 182 | 66.24 | 48.33 |
| | K. Sandhya | Female | 31 | 54 | 156.0 | 183 | 66.72 | 48.75 |
| | B. Sujata | Female | 33 | 52 | 155.0 | 181 | 66.59 | 47.91 |
| | | Female | 40 | 42 | 152.0 | 188 | 64.48 | 50.83 |
| | P. Salmon Raju | Male | 40 | 64 | 157.5 | 184 | 66.69 | 49.16 |
| | G. Narasiah | Male | 46 | 73 | 167.6 | 186 | 62.52 | 50.00 |
| | A. Seetiah | Male | 46 | 85 | 169.2 | 192 | 59.55 | 52.50 |
| | B. Dubbanna | Male | 43 | 88 | 181.2 | 186 | 61.14 | 50.00 |
| | G. Chinniah | Male | 42 | 92 | 179.5 | 189 | 60.38 | 51.25 |
| | R. Ashok | Male | 49 | 63 | 164.2 | 175 | 64.78 | 45.41 |
| 41-50 | V. Kiran | Male | 50 | 77 | 159.3 | 183 | 60.76 | 48.75 |
| | K. Srinivasa Rao | Male | 46 | 62 | 162.6 | 186 | 64.39 | 50.00 |
| | V. Buchi Reddy | Male | 49 | 70 | 160.0 | 190 | 61.24 | 51.66 |
| | D. Gangulu | Female | 45 | 55 | 158.0 | 185 | 60.81 | 49.58 |
| | G. Laxmi | Female | 48 | 58 | 154.0 | 182 | 59.60 | 48.33 |
| | S. Rani | Female | 40 | 53 | 155.0 | 184 | 63.24 | 49.16 |
| | A. Sharada | Female | 45 | 45 | 152.3 | 180 | 63.28 | 47.51 |

**Table 2.** Characteristics of sample used to develop the prediction equation (mean ± s).

| Variable | Ergometer | |
|---|---|---|
| | Male | Female |
| Sample size | 27 | 12 |
| Age (years) | 35 ± 9 | 34 ± 10 |
| Weight (kg)* | 73 ± 11 | 50 ± 6 |
| Height (cm)* | 169 ± 9 | 155 ± 2 |
| Maximum working heart rate (bpm)* | 188 ± 5 | 184 ± 3 |
| $VO_{2max}$ (ml $kg^{-1}$ $min^{-1}$) | 66.5 ± 3.8 | 66.2 ± 4.1 |
| EER (kJ $min^{-1}$)* | 51 ± 2 | 49 ± 1 |

* P < 0.05, difference between the sexes; s = standard deviation

**Table 3.** HR of farm workers with different workloads at 50 rpm speed.

| Workload (N) | Time (min) | Speed (kph) | Distance (km) | Heart rate (bpm) | Heart rate at rest (bpm) | EER (kJ $min^{-1}$) |
|---|---|---|---|---|---|---|
| 9.8 | 1.00 | 23.3 | 0.5 | 118 | 110 | 21.67 |
| | 2.00 | 23.7 | 0.7 | 122 | 104 | 23.33 |
| | 3.00 | 23.5 | 1.1 | 123 | 99 | 23.75 |
| | 4.00 | 23.4 | 1.5 | 127 | 95 | 25.42 |
| | 5.00 | 23.6 | 1.7 | 132 | 92 | 27.50 |
| | 6.00 | 23.7 | 2.2 | 135 | 90 | 28.75 |
| 14.7 | 1.00 | 23.7 | 0.4 | 125 | 122 | 24.58 |
| | 2.00 | 23.6 | 0.6 | 129 | 115 | 26.25 |
| | 3.00 | 23.4 | 0.9 | 134 | 109 | 28.33 |
| | 4.00 | 23.5 | 1.4 | 139 | 104 | 30.42 |
| | 5.00 | 23.2 | 1.6 | 143 | 99 | 32.08 |
| | 6.00 | 23.3 | 2.3 | 147 | 95 | 33.75 |
| 19.6 | 1.00 | 23.3 | 0.4 | 128 | 134 | 25.83 |
| | 2.00 | 23.5 | 0.5 | 133 | 132 | 27.91 |
| | 3.00 | 23.4 | 0.7 | 137 | 125 | 29.58 |
| | 4.00 | 23.6 | 1.2 | 142 | 115 | 31.67 |
| | 5.00 | 23.5 | 1.5 | 146 | 101 | 33.33 |
| | 6.00 | 23.1 | 2.2 | 151 | 98 | 35.42 |

working heart rate (1-6 min) and decreased rest heart rate were 139.5 bpm and 117.5 bpm, respectively graded as 'heavy' and 'moderately heavy' (Zander, 1973). The EER varies from a minimum of 25.83 kJ $min^{-1}$ to a maximum of 35.42 kJ $min^{-1}$. The mean of EER was 30.62 kJ $min^{-1}$ and it is graded as 'heavy' (Zander, 1973).

## Variations of HR of farm workers with different workloads (8.14, 12.3, and 16.4 N) at 60 rpm

The variation of HR of farm workers during cycling Ergometer with different workloads (8.14, 12.3, and 16.4 N) at 60 rpm is shown in Table 4. At 8.14 N workload, the mean values of increased working heart rate (1-6 min) and decreased rest heart rate were 130 and 101.2 bpm, respectively and graded as 'heavy' and 'moderately heavy' (Zander, 1973). The EER varies from a minimum of 22.51 kJ $min^{-1}$ to a maximum of 30.42 kJ $min^{-1}$. The mean of EER was 26.67 kJ $min^{-1}$ and it is graded as moderately heavy (Zander, 1973).

At 12.3 N workload, the mean values of increased working heart rate (1-6 min) and decreased rest heart rate were 133.67 and 109 bpm, respectively and graded

**Table 4.** HR of farm workers with different workloads at 60 rpm speed.

| Workload (N) | Time (min) | Speed (kph) | Distance (km) | Heart rate (bpm) | Heart rate at rest (bpm) | EER (kJ min$^{-1}$) |
|---|---|---|---|---|---|---|
| 8.14 | 1.00 | 26.8 | 1.2 | 120 | 118 | 22.51 |
| | 2.00 | 26.7 | 1.8 | 124 | 114 | 24.17 |
| | 3.00 | 26.5 | 2.4 | 129 | 99 | 26.25 |
| | 4.00 | 26.4 | 2.5 | 133 | 95 | 27.92 |
| | 5.00 | 26.5 | 2.8 | 135 | 92 | 28.75 |
| | 6.00 | 26.6 | 3.5 | 139 | 89 | 30.42 |
| 12.3 | 1.00 | 26.8 | 0.9 | 122 | 135 | 23.33 |
| | 2.00 | 26.7 | 1.4 | 126 | 121 | 25.00 |
| | 3.00 | 26.5 | 1.8 | 131 | 115 | 27.08 |
| | 4.00 | 26.4 | 2.2 | 136 | 98 | 29.17 |
| | 5.00 | 26.5 | 2.7 | 141 | 95 | 31.25 |
| | 6.00 | 26.3 | 3.3 | 146 | 90 | 33.33 |
| 16.4 | 1.00 | 26.9 | 0.8 | 129 | 141 | 26.25 |
| | 2.00 | 26.7 | 1.3 | 134 | 132 | 28.33 |
| | 3.00 | 26.5 | 1.9 | 139 | 120 | 22.50 |
| | 4.00 | 26.5 | 2.5 | 143 | 105 | 32.08 |
| | 5.00 | 26.6 | 2.8 | 148 | 98 | 34.17 |
| | 6.00 | 26.3 | 3.3 | 154 | 95 | 36.67 |

as ‘heavy’ and ‘moderately heavy’ (Zander, 1973). The EER varies from a minimum of 23.33 kJ min$^{-1}$ to a maximum of 33.33 kJ min$^{-1}$. The mean of EER was 28.19 kJ min$^{-1}$ and it is graded as ‘moderately heavy’ (Zander, 1973).

At 16.4 N workload, the mean values of increased working heart rate (1-6 min) and decreased rest heart rate were 141.2 bpm and 115.2 bpm, respectively and graded as ‘heavy’ and ‘moderately heavy’ (Zander, 1973). The EER varies from a minimum of 26.25 kJ min$^{-1}$ to a maximum of 36.67 kJ min$^{-1}$. The mean of EER was 29.95 kJ min$^{-1}$ and it is graded as ‘moderately heavy’ (Zander, 1973).

## Variations of HR of farm workers with different workloads (6.96, 10.5, and 14.0 N) at 70 rpm speed

The variation of HR of farm workers during cycling Ergometer with different workloads (6.96, 10.5, and 14.0 N) at 70 rpm is shown in Table 5. At 6.96 N workload, the mean values of increased working heart rate (1-6 min) and decreased rest heart rate were 132 bpm and 110.2 bpm, respectively and graded as ‘heavy’ and ‘moderately heavy’ (Zander, 1973). The EER varies from a minimum of 23.75 kJ min$^{-1}$ to a maximum of 30.83 kJ min$^{-1}$. The mean of EER was 27.50 kJ min$^{-1}$ and it is graded as ‘moderately heavy’ (Zander, 1973).

At 10.5 N workload, the mean values of increased working heart rate (1-6 min) and decreased rest heart rate were 142 and 118.3 bpm, respectively and graded as ‘heavy’ and ‘moderately heavy’ (Zander, 1973). The EER varies from a minimum of 26.67 kJ min$^{-1}$ to a maximum of 37.08 kJ min$^{-1}$. The mean of EER was 31.66 kJ min$^{-1}$ and it is graded as ‘heavy’ (Zander, 1973).

At 14.0 N workload, the mean values of increased working heart rate (1-6 min) and decreased rest heart rate were 148.67 bpm and 123.67 bpm, respectively and graded as ‘heavy’ and ‘moderately heavy’(Zander, 1973). The EER varies from a minimum of 30.00 kJ min$^{-1}$ to a maximum of 39.17 kJ min$^{-1}$. The mean of EER was 34.44 kJ min$^{-1}$ and it is graded as ‘heavy’ (Zander, 1973).

## Heart rate response and EER of different age groups of farm workers with constant speed (50 rpm) and workload (14.7 N)

From Table 6, the mean values of increased working heart rate (1-12 min) and EER in the age group of 20 to 30 years were 143.58 bpm and 32.32 kJ min$^{-1}$, respectively and it can be graded as ‘heavy’(Zander, 1973).

**Table 5.** HR response of farm worker with different workloads at 70 rpm speed.

| Workload (N) | Time (min) | Speed (kph) | Distance (km) | Heart rate (bpm) | Heart rate at rest (bpm) | EER (kJ $min^{-1}$) |
|---|---|---|---|---|---|---|
| 6.96 | 1.00 | 29.9 | 1.2 | 123 | 129 | 23.75 |
| | 2.00 | 30.3 | 1.8 | 127 | 120 | 25.42 |
| | 3.00 | 30.2 | 2.4 | 131 | 118 | 27.08 |
| | 4.00 | 30.4 | 2.9 | 133 | 102 | 27.92 |
| | 5.00 | 30.3 | 3.5 | 138 | 99 | 30.00 |
| | 6.00 | 30.5 | 3.8 | 140 | 93 | 30.83 |
| 10.5 | 1.00 | 30.2 | 1.00 | 130 | 140 | 26.67 |
| | 2.00 | 30.5 | 1.4 | 135 | 133 | 28.75 |
| | 3.00 | 30.4 | 1.9 | 139 | 128 | 30.41 |
| | 4.00 | 30.6 | 2.6 | 144 | 112 | 32.50 |
| | 5.00 | 30.7 | 2.9 | 149 | 100 | 34.58 |
| | 6.00 | 30.3 | 3.5 | 155 | 97 | 37.08 |
| 14.0 | 1.00 | 30.7 | 0.9 | 138 | 144 | 30.00 |
| | 2.00 | 30.3 | 1.4 | 141 | 139 | 31.25 |
| | 3.00 | 30.5 | 1.8 | 146 | 130 | 33.33 |
| | 4.00 | 30.2 | 2.5 | 151 | 121 | 35.41 |
| | 5.00 | 30.6 | 2.8 | 156 | 109 | 37.50 |
| | 6.00 | 30.4 | 3.3 | 160 | 99 | 39.17 |

**Table 6.** HR and EER of different age groups of farm workers with 50 rpm speed and 14.7 N workload.

| Time (min) | Age group 20-30 | | Age group 31-40 | | Age group 41-50 | |
|---|---|---|---|---|---|---|
| | Heart rate (bpm) | EER (kJ $min^{-1}$) | Heart rate (bpm) | EER (kJ $min^{-1}$) | Heart rate (bpm) | EER (kJ $min^{-1}$) |
| 1.00 | 122 | 23.33 | 118 | 21.67 | 110 | 18.33 |
| 2.00 | 127 | 25.42 | 120 | 22.50 | 118 | 21.67 |
| 3.00 | 133 | 26.67 | 128 | 25.83 | 120 | 22.50 |
| 4.00 | 137 | 28.75 | 134 | 28.33 | 127 | 25.41 |
| 5.00 | 140 | 30.00 | 137 | 29.58 | 130 | 26.67 |
| 6.00 | 143 | 31.25 | 139 | 30.42 | 135 | 28.75 |
| 7.00 | 147 | 32.92 | 143 | 32.08 | 138 | 30.00 |
| 8.00 | 150 | 34.58 | 147 | 33.75 | 141 | 31.25 |
| 9.00 | 153 | 35.83 | 150 | 35.00 | 145 | 32.92 |
| 10.00 | 158 | 38.33 | 154 | 36.67 | 149 | 34.58 |
| 11.00 | 161 | 39.58 | 157 | 37.92 | 152 | 35.83 |
| 12.00 | 165 | 41.25 | 160 | 39.17 | 158 | 38.33 |

In the age group of 31-40, these values were 140.58 bpm and 31.07 kJ $min^{-1}$, respectively and it can be graded as ‘heavy’ (Zander, 1973). In the age group of 41-50, these values were 135.25 bpm and 28.85 kJ $min^{-1}$,respectively and they can be graded as ‘heavy’ and ‘moderately heavy’ respectively (Zander, 1973).

**Table 7.** Comparison of weights and heart rates of farm workers at different speeds.

| Weight (kg) | Speed (rpm) | Heart rate (bpm) | | | Average heart rate (bpm) |
|---|---|---|---|---|---|
| | | 1-4 min | 4-8 min | 8-12 min | |
| 55 | 50 | 133 | 147 | 162 | 147 |
| | 60 | 134 | 148 | 164 | 148 |
| | 70 | 135 | 150 | 165 | 151 |
| 65 | 50 | 131 | 145 | 163 | 146 |
| | 60 | 133 | 146 | 164 | 147 |
| | 70 | 135 | 148 | 165 | 148 |
| 75 | 50 | 130 | 139 | 151 | 140 |
| | 60 | 132 | 141 | 154 | 142 |
| | 70 | 134 | 144 | 159 | 146 |

## Heart rate response of farm workers at different speeds (50, 60 and 70 rpm) and weights (55, 65, and 75 kg)

The effect of heart rate with different age group workers at different weights and speeds are shown in Table 7. The mean values of increased working heart rate (1-12 min) for a weight of 55 kg at three different speeds 50, 60, and 70 rpm are 147.3, 148.67, and 150 bpm, respectively (Table 7). Similarly, the HR values at three different speeds 50, 60 and 70 rpm for a weight of 65 kg are 146, 147, and 148 bpm and for a weight of 75 kg are 140, 142.3, and 145.67 bpm, respectively.

## BMI and PI of the farm workers with different age groups

The mean values of BMI and PI for the farm workers in the age group of 20-30 years were 22.51 and 13.58, respectively (Table 8) and they were graded as 'Normal' and 'Ectomorph' respectively (Garrow, 1981; Florey, 1970). Further, for the 31-40 age group, the mean values of BMI and PI values were 24.05 and 14.57 and they were graded as 'Normal' (Garrow, 1981) and 'Ectomorph' (Florey, 1970), respectively. For the age group of 41-50, the mean values of BMI and PI values were 25.33 and 15.52 and they were graded as 'obese grade I' and 'Ectomorph', respectively (Garrow, 1981; Florey, 1970).

## Statistical analysis

A regression based prediction equation for estimating EER is developed with age, weight, height, heart rate, and maximal oxygen uptake ($VO_{2max}$) as input variables. To develop this equation, a total of 39 form workers sample data was used. The equation was developed separately for men and women as there is a significant difference in weight, height, maximum working heart rate, and EER between the sexes (Table 2). The developed regression based prediction equations are expressed as:

$$EER_{Men} = \left[ \left( -0.0875 \times Age \right) - (0.0383 \times Weight) + (0.000011 \times Height) + (0.3813 \times HR) - (0.2257 \times VO_{2max}) \right] \quad (4)$$

$$EER_{Women} = \left[ \left( -0.0916 \times Age \right) - (0.0401 \times Weight) + (0.0002 \times Height) + (0.3797 \times HR) - (0.2363 \times VO_{2max}) \right] \quad (5)$$

Where $EER_{Men}$ = energy expenditure rate for men (kJ $min^{-1}$), $EER_{Women}$ = energy expenditure rate for women (kJ $min^{-1}$), Age is in years, Weight is in 'kg', Height is in 'cm', HR = heart rate (bpm), $VO_{2max}$ = maximum oxygen consumption rate (ml $kg^{-1}$ $min^{-1}$).

The above equations are developed in Matlab (MATLAB version 7.10.0 [Computer software]. Natick, MA, MathWorks.) software. For inner validation, both the models for women and men farm workers were tested on an independent sample of total 39 participants. The scatter plots between observed and regression model predicted EER (kJ $min^{-1}$) for men and women is shown in

**Table 8.** BMI and PI of the farm workers with different age groups.

| Age group (years) | Name of the operator | Sex | Age (years) | Weight (kg) | Height (m) | BMI | PI |
|---|---|---|---|---|---|---|---|
| | N. Ashok Kumar | Male | 27 | 62 | 1.702 | 21.40 | 12.58 |
| | N. Surya Prakash | Male | 25 | 68 | 1.702 | 23.47 | 13.79 |
| | S. Venkat Reddy | Male | 26 | 52 | 1.575 | 20.96 | 13.31 |
| | K. Tirupati | Male | 22 | 70 | 1.685 | 24.65 | 14.63 |
| | R. Prasad | Male | 29 | 59 | 1.63 | 22.21 | 13.62 |
| | K. Sathyanarayana | Male | 27 | 72 | 1.79 | 22.47 | 12.55 |
| 20-30 | V. Shankar | Male | 26 | 80 | 1.65 | 29.38 | 17.81 |
| | B. Bhuchanna | Male | 22 | 92 | 1.72 | 31.10 | 18.08 |
| | D. Rajayya | Male | 30 | 77 | 1.85 | 22.50 | 12.16 |
| | A. Narsakka | Female | 20 | 45 | 1.55 | 18.73 | 12.08 |
| | Ch. Manga | Female | 21 | 48 | 1.57 | 19.47 | 12.40 |
| | N. Bhulaxmi | Female | 25 | 42 | 1.52 | 18.18 | 11.96 |
| | G. Vani | Female | 30 | 44 | 1.56 | 18.08 | 11.59 |
| | N. Thirupathayya | Male | 33 | 56 | 1.575 | 22.57 | 14.33 |
| | S. Srinivasa Rao | Male | 34 | 62 | 1.575 | 24.99 | 15.87 |
| | K. Srinu | Male | 34 | 74 | 1.676 | 26.34 | 15.72 |
| | Ch. Kutumba Rao | Male | 39 | 88 | 1.823 | 26.48 | 14.53 |
| | M. Mallikarjuna | Male | 32 | 90 | 1.762 | 28.99 | 16.45 |
| | N. Veeranna | Male | 36 | 76 | 1.652 | 27.85 | 16.86 |
| 31-40 | U. Gangaram | Male | 38 | 63 | 1.812 | 19.19 | 10.59 |
| | A. Daniel | Male | 33 | 75 | 1.75 | 24.49 | 13.99 |
| | U. Sukumar | Male | 32 | 70 | 1.68 | 24.80 | 14.76 |
| | M. Padmaja | Female | 30 | 60 | 155.0 | 24.97 | 16.11 |
| | K. Sandhya | Female | 31 | 54 | 156.0 | 22.19 | 14.22 |
| | B. Sujata | Female | 33 | 52 | 155.0 | 21.64 | 13.96 |
| | | Female | 40 | 42 | 152.0 | 18.18 | 11.96 |
| | P. Salmon Raju | Male | 40 | 64 | 157.5 | 25.80 | 16.38 |
| | G. Narasiah | Male | 46 | 73 | 167.6 | 25.99 | 15.51 |
| | A. Seetiah | Male | 46 | 85 | 169.2 | 29.69 | 17.55 |
| | B. Dubbanna | Male | 43 | 88 | 181.2 | 26.80 | 14.79 |
| | G. Chinniah | Male | 42 | 92 | 179.5 | 28.55 | 15.91 |
| | R. Ashok | Male | 49 | 63 | 164.2 | 23.37 | 14.23 |
| 41-50 | V. Kiran | Male | 50 | 77 | 159.3 | 30.34 | 19.05 |
| | K. Srinivasa Rao | Male | 46 | 62 | 162.6 | 23.45 | 14.42 |
| | V. Buchi Reddy | Male | 49 | 70 | 160.0 | 27.34 | 17.09 |
| | D. Gangulu | Female | 45 | 55 | 158.0 | 22.03 | 13.94 |
| | G. Laxmi | Female | 48 | 58 | 154.0 | 24.46 | 15.88 |
| | S. Rani | Female | 40 | 53 | 155.0 | 22.06 | 14.23 |
| | A. Sharada | Female | 45 | 45 | 152.3 | 19.40 | 12.74 |

Figure 6a and b, respectively. The fit line equations ($y = a_0x+a_1$) are shown in Figures 6a and b along with the coefficient of determination ($R^2$) values. There is a quite good agreement between the observed and predicted EER with $R^2$ values as 0.738 and 0.645 for both the men and women, respectively.

## Conclusions

From the results of experimental investigation the following conclusions were drawn:

1. The farm workers of 20-30 years age group have

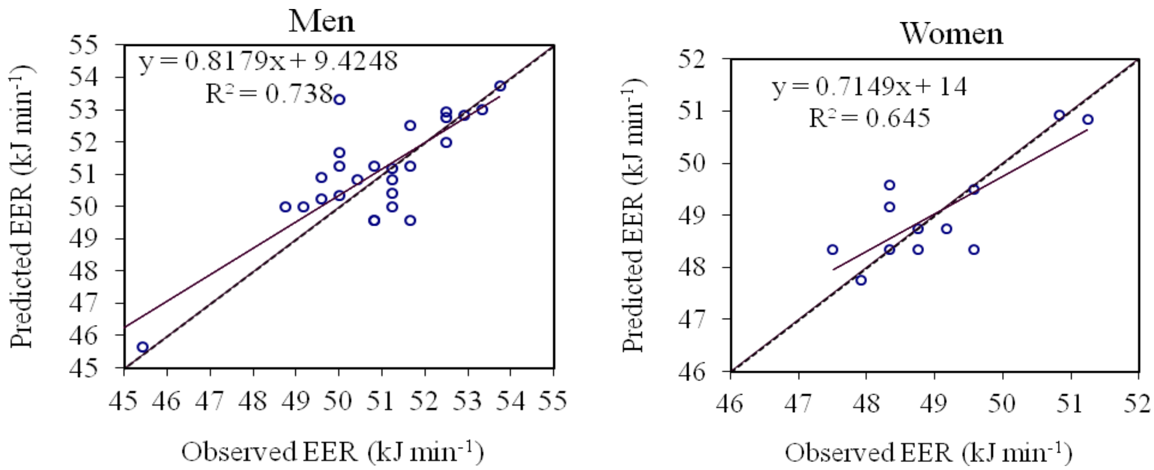


**Figure 6.** Scatter plots between observed and predicted EER for a) Men and b) women farm workers.

developed a maximum working heart rate as compared to other age groups of 31-40 and 41-50 years.

2. The EER for all the farm workers obtained was graded as ‘Moderately Heavy’.

3. As the workload values increases, the heart rates of the farm workers increased.

4. The BMI of age groups 20-30 and 31-40 years were considered as ‘Normal’ and 41-50 were considered as ‘Obese grade I’.

5. The PI of all age groups, that is, 20-30, 31-40 and 41-50 years were ‘Ectomorph’ in nature.

6. The regression based equations were developed and validated for both the men and women in predicting EER from age, weight, height, heart rate, and maximal oxygen uptake ($VO_{2max}$) as input variables.

## Conflict of Interest

The author has not declared any conflict interest.

## ACKNOWLEDGEMENTS

The authors are very much thankful to the farm workers for their participation and cooperation during conducting experiment. The authors would like to express their gratitude to the College of Agricultural Engineering Instrumentation Lab, Bapatla, Andhra Pradesh, India for providing required equipment to carry out this study.

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academicJournals

Vol. 10(28), pp. 2680-2686, 9 July, 2015
DOI: 10.5897/AJAR2015.10015
Article Number: 2681FDC53954
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

*Full Length Research Paper*

# Relationship of soil quality and Vitexicarpine content in the leaves of *Vitex trifolia* L.

**Eko Hanudin[1*], Yosi Bayu murti[2], Mifhadi Setiawan[1] and Zullies Ikawati[3]**

[1]Laboratory of Soil Chemistry and Fertility, Faculty of Agriculture, UGM Yogyakarta 55281, Indonesia.
[2]Laboratory of Pharmaceutical Biology, UGM Yogyakarta 55281, Indonesia.
[3]Laboratory of Pharmacology and Toxicology, Faculty of Pharmacy, UGM Yogyakarta 55281, Indonesia.

Received 10 June, 2015; Accepted 18 June, 2015

**Vitexicarpine has been known as a marker compound observed in the leaves of *Vitex trifolia* L. An explorative research was conducted to find out relationship of soil quality and vitexicarpine content in the leaves of *V. trifolia* L. The plant was found in the some districts in Central Java (Surakarta) and Yogyakarta (Kulon Progo). Soil types where the plant grew are Entisols, Inceptisols and Andisols. Based on soil characteristics, the soil quality indexes for each soil type were calculated by referring to Karlen methods. The results indicated that soil quality index and vitexicarpine content in the leaves of *V. trifolia* L had a negative correlation. Lower the soil quality tended to result in higher content of vitexicarpine. Soil quality index of Andisols from Karangpandan-Surakarta was 0.991 and vitexicarpine content in the leaves was only 0.6%, but in case of Entisols the soil quality index was 0.274 and vitexicarpine content was able to reach around 0.82%. Inceptisols quality index was an intermediate between Andisols and Entisols. However, individually nutrient indicated a positive correlation with vitexicarpine content. Higher Nitrogen and Magnesium content in the soil resulted higher vitexicarpine content in the leaves. This may related to the role of the both nutrientin biosynthesis of vitexicarpine.**

**Key words:** Entisols, Inceptisols, Andisols, aglycone vitexicarpine, and glycoside vitexicarpine.

## INTRODUCTION

*Vitex trifolia* L. was found in several countries such as southern Africa, Madagascar and Mauritius to Afghanistan, India, Sri Lanka, Burma (Myanmar), Indo-China, southern China, Japan, Thailand, throughout the Malesian region, south to northern Australia, east to New Caledonia and Indonesia (Capareda, 1999). *V. trifolia* L. is one of thousands medical plants that grow in Indonesia. The plant was known as an annual plant which has many beneficial. Until now, information about the marker compound Vitexicarpine in the medical plant *V. trifolia* L. is still scarce. Some Indonesian traditional therapists believe that the leaves of *V. trifolia* could be used as a medicine for antiasthma, anti-allergy and anticancer. In India, Murugan and Mohan (2012) used the plant as an antifeeding activity against the insect pest *Spodoptera frugiperda,* antifungal activity, and antibacterial activities.

The leaves of *V. trifolia* contain a volatil oil composed of

*Corresponding author. E-mail: ekohanudin@ugm.ac.id, Fax: +62274548814.

sesquiterpen, terpenoide, ester compound, alcaloid (vitrisin), glycoside flavon (artemetin and 7-desmetil artemetin) and non flavonoid friedelin components, ß-cytosterol, glucoseand hydrocarbon compound which has analgesic and anti allergy (Alam et al., 2002). Vitexicarpinis known as a pharmacologic active component in the leaves of *V. trifolia* and suitable as a marker compound (Alam et al., 2002; Ikawati et al., 2001). Therefore, *V. trifolia* L. is a prospective plant to be developed as medical plant in an industry scale. For the purpose, the plant should be grown in a good environment and soil quality (Maheswari, 2002). Information about relationship between a marker compound in the plant and soil characteristics is still so scarce, also still rare information on the relationship between the content of a marker compound in the plant with the soil characteristics where the plants grow. The main objective of this research is to find out relationship of soil quality index and vitexicarpine content in the leaves of *V. trifolia*.

## MATERIALS AND METHODS

This research was conducted in 7 locations, at sub district of Tawangmangu and Karangpandan. The two locations were situated in District of Surakarta (Province of Central Java) and the five were situated in district of Kulon Progo (Province of Yogyakarta). Soil types where the *V. trifolia* grew on it were described. Based on diagnostic horizon characteristics of the both soil profile from Tawangmangu and Karangpandan could be classified as Andisols (according to soil taxonomy USDA). Whereas, soil types collected from the five locations situated in Kulon Progo were two Entisols and three Inceptisols. Soil physical and chemical analyses was done for soil texture (sand, silt and clay fractions were determined by pipette method), porosity (n), bulk density (wax method), pH (electrode pH-meter), organic carbon (Walkey and Black), total nitrogen (Kjeldahl method), available phosphorous (Olsen method), K-available, Ca-available and Mg-available (extracted by $NH_4OAc$, pH 7 and measured by AAS). Soil quality index was calculated based on the soil physico-chemical properties by referred to Karlen et al. (1996). Procedure for Vitexicarpine analysis was done as follow: dried leaves extracted with ethyl acetate, concentrated *in vacuo* and further analyzed for aglycone vitexicarpine. The residue was dried from ethyl acetate, extracted with methanol 50% in water and concentrated *in vacuo*. Methanol extract was hydrolyzed in acidic condition (HCl 10%, 80°C, 1 h) and extracted by ethyl acetate. The ethyl acetate fraction was concentrated *in* vacuo and further analyzed the vitexicarpine as glycoside vitexicarpine. Vitexicarpine content analyzed by thin layer chromatography with stationary phase silica gel F254 (Merck), mobile phase n-hexane:ethyl acetate (2:1 v/v), and 8 cm migration distance. The chromatogram was scanned in TLC Scanner 3 (Camag) at wavelength 346 nm (Alam et al., 2002).

## RESULTS AND DISCUSSION

### Physical and chemical properties of the soils

Physical and chemical properties of soils under *V. trifolia* L. plantation are presented in Table 1. In general, the authors observed three orders of soils, namely: Andisols, Incepstisols, and Entisols. The soil physical properties such as texture (proportion of sand, silt and clay), bulk density, particles density and porosity were in a range of sandy clayey loam–loam, 0.78-1.2 gcm$^{-3}$, 1.17-2.52 gcm$^{-3}$ and 34.47-53.83%, respectively. The soil reaction could be categorized as slightly acid-neutral (pH-$H_2O$, 6.04-6.6). The concentration of N, P, K and C-organic (except for Andisols in a high rate) in all of the soil types were categorized as a low rate. In case of $Ca^{2+}$, $Mg^{2+}$ and Fe content was categorized as medium-high rate (SRC, 2009).

### Vitexicarpine content in the leaves of *V. trifolia*

Analysis of *V. trifolia* L. leaves indicated that vitexicarpine content in the leaves was a wide variation. It is likely to related to leaves maturation and location where the leaves collected (presented in Figure 1). Aglycone vitexicarpine content is higher in young leaves (0.36-0.5%) than older leaves (0.22-0.33%). As levels of free forms (Aglyconevitexicarpine), levels of bound forms (glycoside vitexicarpine) also showed similar results. Young leaves had a higher concentration (0.32 to 0.53%) than older one (0.24-0.42%). This result is surprising considering the higher vitexicarpine content was found in the leaves with low maturation level.

Proportion between the aglycone vitexicarpine and glycoside vitexicarpine content was not depend on the leaves maturation level (Murti et al., 2009). In general, the content of the glycoside vitexicarpine was higher than the aglycone vitexicarpine. This will lead to the consequences of selecting the optimal solvent for extraction of the *V. trifolia* L. leaves.

Comparing among the sample locations indicated that the total vitexicarpine content in the leaves was so various (Table 3), in the young and old leaves were observed at a range of 0.73-1.03% and 0.46-0.75%, respectively. In average, the total vitexicarpine concentration in the young leaves was significantly higher than the old one. Therefore, for the purpose of standardizing the leaves extract of *V. trifolia* would be better if in the process of harvesting young leaves have just taken. Among the 7 locations indicated that there are 3 locations where the vitexicarpine content in the leaves was higher than the other places, namely: Kulon, Progo (only for KP-1 and KP-2) and Tawangmangu. Thus the three locations were fit for use as a raw materials source of the *V. trifolia* leaves extract. Alam et al. (2002) proposed the chemical structure of the vitexicarpine presented in Figure 2.

### Soil quality index (SQI) and Vitexicarpine content

Soil quality is the capacity of a soil to function within

**Table 1.** Selected physical and chemical properties of the soils studied.

| Soil physical-chemical properties | Soil types | | | | | | |
|---|---|---|---|---|---|---|---|
| | Ent KP-1 | Ent KP-2 | Inc KP-3 | Inc KP-4 | Inc KP-5 | And-Tm | And-Kpd |
| Mineral Fractions: Clay (%) | 12.23 | 11.54 | 18.07 | 30.36 | 22.45 | 9.81 | 35.38 |
| Silt (%) | 14.36 | 17.79 | 22.56 | 24.11 | 23.66 | 37.24 | 36.65 |
| Sand (%) | 73.41 | 70.66 | 59.37 | 45.49 | 53.88 | 52.94 | 27.96 |
| Texture class | Sandy Loam | Sandy Loam | Sandy Loam | Sandy Clayey Loam | Sandy Clayey Loam | Loam | Sandy Clayey Loam |
| BD (g $cm^{-3}$) | 1.11 | 1.15 | 1.14 | 1.20 | 1.14 | 0.78 | 0.78 |
| PD (g $cm^{-3}$) | 2.40 | 2.44 | 2.43 | 2.52 | 2.48 | 1.17 | 1.16 |
| Porosity (%) | 53.75 | 52.81 | 53.15 | 52.40 | 53.83 | 34.47 | 32.20 |
| pH-$H_2O$ | 6.5 | 6.53 | 6.50 | 6.6 | 6.46 | 6.04 | 6.05 |
| pH-KCl | 5.3 | 4.98 | 4.93 | 6.1 | 5.35 | 5.67 | 4.94 |
| pH-NaF | - | - | - | - | - | 9.92 | - |
| N-total (%) | 0.06 | 0.09 | 0.07 | 0.08 | 0.08 | 0.38 | 0.25 |
| P-Olsen (ppm) | 7.63 | 7.97 | 5.43 | 6.37 | 6.10 | 2.71 | 5.84 |
| K ($cmol^+Kg^{-1}$) | 0.17 | 0.20 | 0.13 | 0.97 | 0.24 | 0.21 | 1.19 |
| Ca ($cmol^+Kg^{-1}$) | 18.47 | 23.21 | 20.04 | 21.37 | 19.15 | 9.65 | 14.88 |
| Mg ($cmol^+Kg^{-1}$) | 13.34 | 14.44 | 13.74 | 10.57 | 12.44 | 2.21 | 9.67 |
| Fe-avl (ppm) | 20.64 | 33.47 | 41.60 | 24.98 | 49.28 | 42.97 | 72.68 |
| C-organic (%) | 0.17 | 1.17 | 0.75 | 0.65 | 1.03 | 11.97 | 3.30 |
| Org. Matter (%) | 0.30 | 2.01 | 1.29 | 1.11 | 2.09 | 20.65 | 5.69 |
| CEC ($cmol^+Kg^{-1}$) | 35,31 | 49,00 | 38,08 | 40,41 | 35,51 | 32,24 | 28.65 |

Note: And-Tm: Andisols-Tawangmangu, Ent KP: Entisols Kulon Progo, Inc KP: Inceptisols Kulon Progo, Inc-Kpd: Inceptisols Karangpandan, BD: Bulk Density, PD: Particle Density.

ecosystem boundaries to sustain biological productivity, maintain environmental quality, and improve the health of plants and animals (Doran and Parkin, 1994; Karlen et al., 1996). Soil quality index is an index based on the value and weight of each indicator of soil quality. Soil quality indicators were selected from properties that show the capacity of the soil functions that determine the level of soil fertility. Indicators of soil quality are the nature, characteristics or physical processes, chemical, and biological soil that can describe the condition of the soil (Doran and Parkin, 1994; Andrews et al., 2004). Based on the calculation of the soil quality index of each research area are presented in Table 2.

According to Wander et al. (2002) based on scoring functions indicated soil quality rate for a particular land use determined from 0-1. Soil quality index values closer to the value of one the better soil quality. Based on Table 2, it was known that the soil quality index of the seven research sites could be categorized at a range of low to very good rating. Soil samples from Kulon Progo 1 and 2 could be classified as Entisols order and have SQI values of 0.274 and 0.315, respectively and the both value could be categorized as a low soil quality rate. While the soil samples from the Kulon Progo 3, 4 and 5 could be classified as Inceptisols order and have SQI values of 0.452, 0.580 and 0.541 respectively, and all the three value could be categorized as a medium soil quality rate. Soil which has a very good rate in the soil quality is Andisols orders found in Tawangmangu and Karangpandan (0.818 and 0.991, respectively).

Based on Table 3, it is known that the location of study has a soil quality index and vitexicarpine content in the leaves of *V. trifolia* diverse. The relationship between soil quality index and

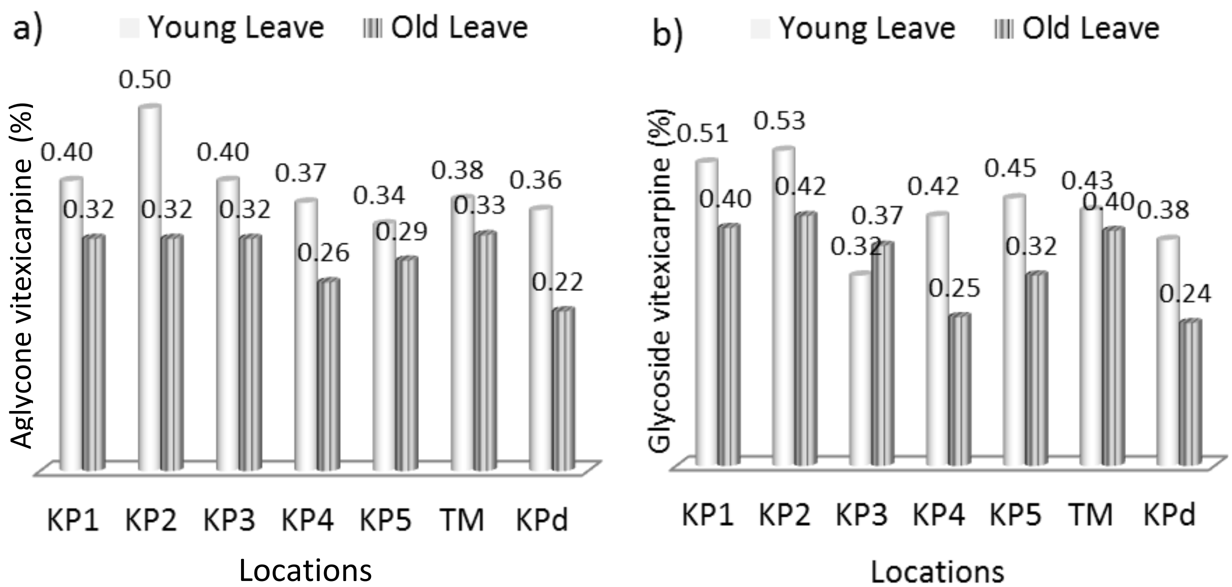


**Figure 1.** Distribution of vitexicarpine type (%) in young and old leaves of *V. trifolia*: a). aglycone vitexicarpine, and b). glycoside vitexicarpine.

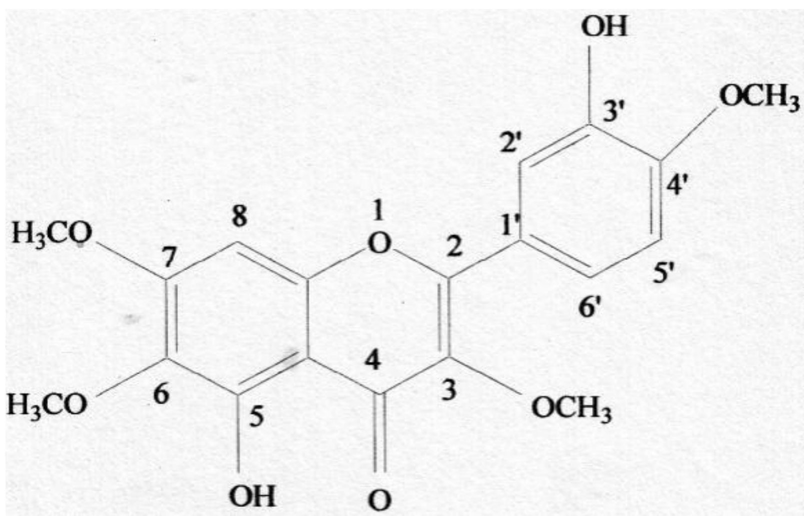


**Figure 2.** Chemical structure of vitexicarpin proposed by Alam et al. (2002).

**Table 2.** Soil quality index (SQI) from the area studied.

| Soil sample location | Soil quality index | SQI rate |
|---|---|---|
| KP-1 | 0.274 | Low |
| KP-2 | 0.315 | Low |
| KP-3 | 0.452 | Medium |
| KP-4 | 0.580 | Medium |
| KP-5 | 0.541 | Medium |
| TM | 0.818 | Very Good |
| KRP | 0.991 | Very Good |

Note: KP: Kulon Progo, Tm: Tawangmangu, Kpd: Karangpandan.

**Table 3.** Relationship of soil quality index and total vitexicarpine concentration in young and old leaves of *V. trifolia*.

| Locations | Soil quality index | Total Vitexicarpine content (%) | | Average (%) |
|---|---|---|---|---|
| | | Young leaves | Old leaves | |
| KP-1 | 0.274 | 0.90 | 0.73 | 0.82 |
| KP-2 | 0.315 | 1.03 | 0.75 | 0.89 |
| KP-3 | 0.452 | 0.73 | 0.69 | 0.71 |
| KP-4 | 0.580 | 0.79 | 0.51 | 0.65 |
| KP-5 | 0.541 | 0.78 | 0.60 | 0.69 |
| TM | 0.818 | 0.81 | 0.72 | 0.77 |
| KRP | 0.991 | 0.74 | 0.46 | 0.60 |

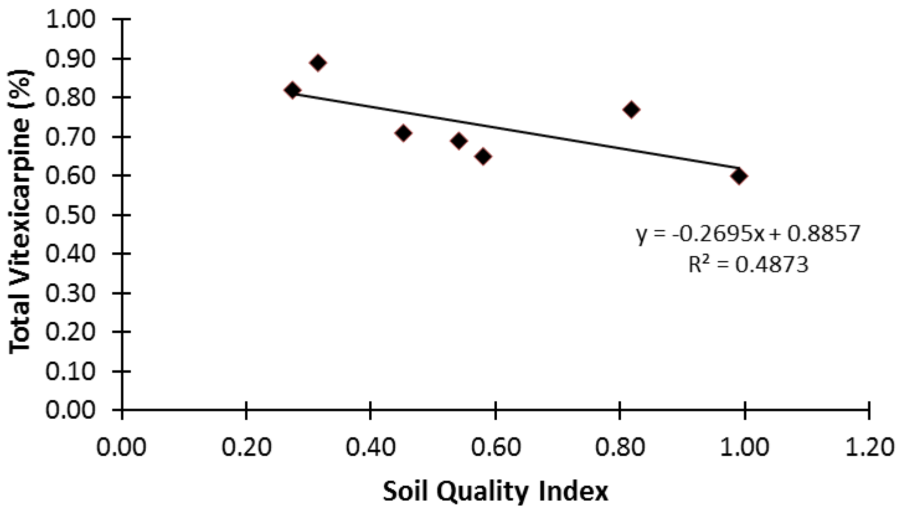


**Figure 3.** Relationship between soil quality index and vitexicarpine concentration.

vitexicarpine concentration in the leaves of *V. trifolia* was a negative correlation (Figure 3). This means that the higher the soil quality index is the vitexicarpine concentration in the leaves of *V. trifolia* relatively is lower. This is due to the plant has a capability for survival through a certain metabolism mechanism in the plant cell if under a high environmental pressure. Soil quality index of Andisols from Karangpandan-Surakarta was 0.991 and vitexicarpine content in the leaves was only 0.6%, but in case of Entisols, the soil quality index was 0.274 and vitexicarpine content was able to reach around 0.82%. Inceptisols quality index was an intermediate between Andisols and Entisols.

In general, it has been known that soil is an important factor controlling the plant growth. Nutrient availability in soil is needed to produce the secondary metabolite compounds in the plant. There are several soil nutrients has an important role in synthesis of vitexicarpine, such as: nitrogen, magnesium and iron. Nitrogen and magnesium has an important role in synthesis of flavonoid compound (Torsell, 1997). Nitrogen also has an important role in synthesis of amino acid and protein in the plant (Epstein and Bloom, 2005). In the synthesis process of the secondary metabolite, the role of nitrogen as a *building material,* especially for synthesis of amino acid (tyrosine and phenylalanine) through biosynthetic pathway of shikimic acid inform of phenile propane ($C_6$-$C_3$) (Winkel, 2006).

Relationship between N-total in the soil and vitexicarpine concentration in the young and old leaves of *V. trifolia* L was presented at Figure 4. Based on the Figure 4, nitrogen in the soil has a positive correlation with vitexicarpine concentration in the leaves of *V. trifolia* L.

It is well known that N is a mobile nutrient in the plant, so the N content in the young leaves is higher than the olderone (Epstein and Bloom, 2005). This has become one of the causes of vitexicarpine concentration in young leaves was higher than in older leaves. Concentration of N in Entisols and Inceptisols were observed at range of 0.06-0.09%. According to soil chemical analysis criteria (SRC, 2009), the range of the value could be categorized as a very low rate, while in Andisols were observed at range of 0.25-0.38 (medium) (Table 1).

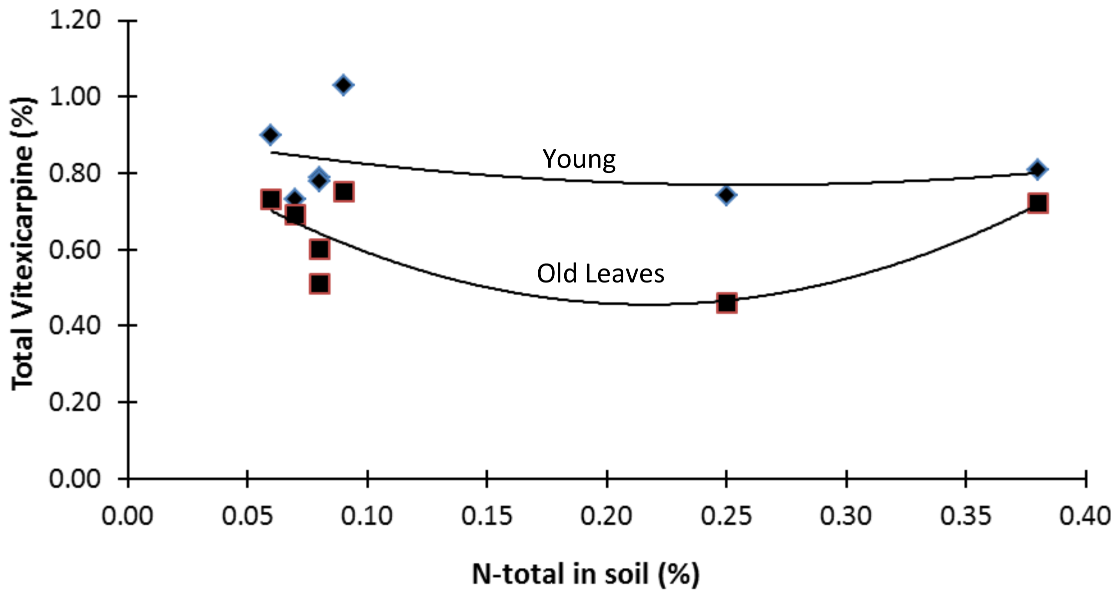


**Figure 4.** Relationship between N-total in the soil and vitexicarpine concentration in the young and older leaves of *V. trifolia* L.

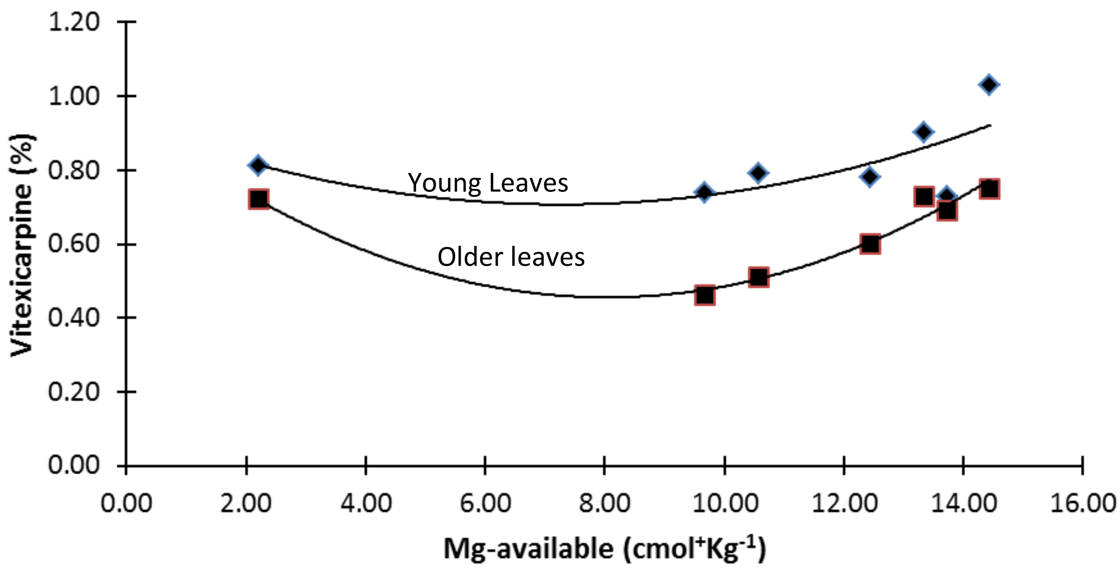


**Figure 5.** Relationship between Mg-available in the soil and vitexicarpine concentration in the young and older leaves of *V. trifolia* L.

Magnesium (Mg) also an important macronutrients required by plant as a nucleus of chlorophyl molecule. The molecule has an important role in photosynthesis, synthesis of fatty acid and oil in the plant (Epstein and Bloom, 2005). In the synthesis of secondary metabolite, the role of Mg as a catalyst for biosynthetis pathway from malonic acetate to Acetyl CoA ($C_2$) and contributed in increasing the vitexicarpine in the leaves of *V. trifolia* L. Combination of the both biosynthetic pathway of malonic acetate and shikimic acid resulted in flavonoid compounds. The flavonoid compound should undergo a flavonoid methyltransferase process with $Mg^{2+}$ ion and SAM as catalyst and finally resulted in the vitexicarpine compound (Torsell, 1997). Relationship between Mg-available in the soil and vitexicarpine concentration in the young and older leaves of *V. trifolia* L. was prsented in Figure 5. Based on the figure, magnesium in the soil has a positive correlation with vitexicarpine concentration in the leaves of *V. trifolia* L.

Magnesium is a mobile nutrient in the plant even not as mobile as nitrogen, but Mg in the older leaves will be translocated to the young leaves. That is why the Mg content in the young leaves is higher than the older one (Epstein and Bloom, 2005). This may be related to the positive correlation between Mg and vitexicarpine concentration in the leaves of *V. trifolia* L. and also resulted in the vitexicarpine concentration in young leaves was higher than in older one.

Concentration of Mg in Andisol, Entisols and Inceptisols were observed at range of 2.21-9.67, 13.34-14.44 and 10.57-13.74%, respectively. According to soil chemical analysis criteria (CSR, 2009), the range of the value could be categorized as a high-very high rate. A high availability of Mg in the soil resulted in the Mg content in the plant also become high and may stimulate for formation of Clorophyl in turn, the production of oil fatty acid and metabolite secondary (such as vitexicarpine) also increased (Marschner, 1995; Merhaut, 2007).

## Conclusions

A negative correlation was observed for the soil quality index and vitexicarpine content in the leaves of *V. trifolia*. Lower the soil quality tends to result in higher content of vitexicarpine. Soil quality index of Andisols from Karangpandan-Surakarta was 0.991 and vitexicarpine content in the leaves was only 0.6%, but in case of Entisols was 0.274 and vitexicarpine content was able to reach around 0.82%. Inceptisols quality index was an intermediate between Andisols and Entisols. However, individually nutrient indicated a positive correlation with vitexicarpine content. Higher Nitrogen and Magnesium content in the soil resulted higher vitexicarpine content in the leaves. This may related to the role of the both nutrient in biosynthesis of vitexicarpine.

## Conflict of Interest

The authors have not declared any conflict of interest.

## ACKNOWLEDGEMENT

The authors would like to give thanks to Indonesia Minister of Research and Technology that supported with the fund for conducting this research.

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academicJournals

Vol. 10(28), pp. 2687-2694, 9 July, 2015
DOI: 10.5897/AJAR2015.9499
Article Number: D6812B053956
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

*Full Length Research Paper*

# Nitrogen and sulfur absorption by quality protein maize (QPM) maize as affected by interaction between nitrogen and sulfur fertilizers at Samaru, Zaria, Nigeria

**M. M. Jaliya[1]*, M. K. Othman[1], B. M. Sani[1], B. A. Babaji[2], Y. A. Sani[1] and A. M. Zongoma[3]**

[1]National Agricultural Extension and Research Liaison Services (NAERLS), Ahmadu Bello University (ABU) Zaria, Samaru, Zaria, Nigeria.
[2]Department of Agronomy, Ahmadu Bello University Zaria, Samaru, Zaria, Nigeria.
[3]Department of Biological Sciences, Ahmadu Bello University Zaria, Samaru, Zaria, Nigeria.

Received 12 January, 2015; Accepted 18 June, 2015

**Field trials were conducted in 2006, 2007 and 2008 wet seasons, at the Institute for Agricultural Research Farm, Samaru in the Northern Guinea savanna of Nigeria to determine the effect of interaction between Nitrogen and Sulfur fertilizers on up-take of Nitrogen and Sulfur by quality protein maize (QPM). Treatments consisted of four rates each of nitrogen (0, 60,120 and 180 kg N/ha) and sulfur fertilizer (0, 5, 10 and 15 kg S/ha) and two QPM varieties (Obatampa and EV – 99), laid out in a split plot design with variety and nitrogen in the main plots and sulfur in the sub plots and replicated three times. The result of this study showed that there was a significant effect of interaction between nitrogen and sulfur on up-take of both nitrogen and sulfur by quality protein maize (QPM). Higher up-take of N was influenced by the interaction of 120 kg N/ha and 15 kg S/ha in 2006, 0 kg N/ha and 5 kg S/ha in 2007, 120 kg N/ha and 0 kg S/ha in 2008. When sulfur up-take was observed, interaction between 180 kg N/ha and 5 kg S/ha in 2006, 0 kg N/ha and 15 kg S/ha in 2007, 180 kg N/ha and 10 kg S/ha in 2008 produced higher up-take by the crop. It could be concluded from this study that interaction between nitrogen and sulfur fertilizers significantly influenced up-take of N and S nutrients by QPM. However, level of influence varies between different fertilizer rate's interactions.**

**Key words:** Nitrogen, Sulfur, interaction, quality protein maize (QPM).

## INTRODUCTION

Quality protein maize is more nutritious than conventional maize. This is due to the fact that its amino acid profile contains two amino acids namely lysine and tryptophan in their endosperm which are lacking in the amino acid profile of conventional maize (Paiva et al., 1991). This was evident from a trial by Akuamoa-Boateng (2002) where 422 children were fed with either QPM or normal maize in Ghana. The result indicated that children in the QPM group had significantly fewer sick days and less stunting, compared to children in the normal maize group.

*Corresponding author. E-mail: jaliyamuhammad@yahoo.com, jaliyamm55@gmail.com, Tel: 08036480353.

As a result of the improvement, the QPM's amino acid profile gives a good balance of total essential amino acids and has an amino acid score adjusted for digestibility of 67% compared to 31.0 and 33.0% values found for dent and flint maize, respectively (Zarkadas et al., 1995*)*. In normal maize, the primary amino acid deficiencies include lysine, theonine and tryptophan (Zarkadas et al., 1995)*.* QPM may supply 70 to 73% of human protein requirement, compared with 46% for normal maize. The biological value of the protein (a value that measures how well the body can absorb protein) is 80% for QPM compared with 40 to 57% for normal maize and 86% for eggs (Bressani, 1992). The protein of QPM has 90% of the relative value (RV) of milk compared to 40% for normal maize (Badu-Apraku and Fontem-Lum, 2010).

Nitrogen is a major plant nutrient for growth and makes up 1 to 4% of dry matter of plants (Anon., 2000). It is a component of protein and nucleic acids and when N is sub-optimal; growth is reduced (Hague et al., 2001). Nitrogen is the most limiting nutrient in the savanna soils where the soils are predominantly coarse textured and characteristically low in organic matter (Anon., 1989). Its deficiency is usually recognized first by pale green or yellowish green colour of the leaves, followed by premature necrosis of the older leaves. Where soils are rich in organic matter, such as where the land has just been cleared after a long fallow, a fair amount of nitrogen would be made available to crops through the decomposition of the organic matter (Anon., 1989). Based on the importance of nitrogen as mentioned above, it was chosen as one of the factors of the treatment. Sulfur is the fourth major nutrient after N, P and K. It is a constituent of the essential amino acids lysine and tryptophan. On the average, maize crop absorbs as much S as it absorbs P. When S is deficient in soil, full yield potential of the crop cannot be realized regardless of other nutrients even under good crop husbandry practices (Tandon, 1989). Deficiency of S is likely to be widespread in Africa, especially in the savanna regions, where annual bush burning results in losses of sulfur to the atmosphere as Sulfur dioxide ($SO_2$) (Tandon, 1989). The investigation was therefore, aimed at evaluating the effects of nitrogen and sulfur on quality protein maize (QPM) varieties with respect to growth and yield performance, nutrient uptake and protein content of grains. The specific objectives were:

i. To determine the optimum rate of N on growth and yield of QPM,
ii. To determine the optimum rate of S on growth and yield of QPM, varieties,
iii) To determine which of the two QPM varieties is best for northern Guinea Savanna agro ecological zone.

## MATERIALS AND METHODS

The experiment to evaluate the uptake of nitrogen and sulfur by two quality protein maize varieties was conducted for three years during the wet season of 2006, 2007 and 2008 at Samaru, Zaria (11º 11′ N; 07º 38′ E and 686 m above sea level), located in the northern Guinea Savanna zone of Nigeria. Rainfall normally establish as between mid-May to early June and peaks in July/August. Annual precipitation ranges between 800 to 1300 mm, with an average of 1100 mm (IAR, 2009).

The experiment was laid out in split plot design with nitrogen and maize variety in the main plot and sulfur in the subplot. The treatments consisted of two open pollinated QPM varieties (Obatanpa and EV – 99), four rates each of nitrogen (0, 60, 120 and 180 kg N/ha) using urea (46%N) and sulfur (0, 5, 10, and 15 kg S/ha) using potassium sulfate (1%S) to evaluate effects of nitrogen and sulfur on quality protein maize (QPM) varieties with respect to growth and yield performance, nutrient uptake and protein content of grains. The experiment was replicated three times. Borders between plots within a replication were separated by one metre spacing and between replications by spacing of 1.5 m. Gross plot size was 6 ridges that is, 4.5 m by 2.5 m, giving an area of 11.25 $m^2$, while net plot size was 4 ridges that is, 3.0 m by 1.5 m with an area of 4.5 $m^2$.

The two varieties used for the trials were open pollinated quality protein maize namely Obatanpa and EV-99; both were sourced from Institute for Agricultural Research Ahmadu Bello University Zaria. Obatanpa is a non tillering variety, erect, medium maturing with 106 to 110 days to physiological maturity. The plant height is 150 to 245 cm, while the plant colour is green. Potential grain yield of Obatanpa is 5.8 t/ha (Ado et al., 2009). The seed characteristics shows that the row arrangement is straight with 14 to 18 rows per cob, the kernel is white and kernel type is dent/flint. Obatanpa has high essential amino acids, lysine (3.9%) and tryptophan (1.1%) about 56% higher than normal maize with protein content of 10 to 12%. In addition to high yield, it is tolerant to striga infestation, stem borer and maize streak virus (MSV). EV – 99 is medium maturing at about 58 days to mid-silking with 170 cm in height, white seeded kernels. Adapted to lowland tropics with days to maturity of 90 to 95 days, high yield with potential yield of 5.5 t/ha. It is tolerant to *Striga hermonthica* and resistant to maize streak virus (Ado et al., 2009).

Soils were randomly sampled from several points spread within the experimental site before land preparation each year, at the depth of 0 to 30 cm and a composite sample was taken, dried, ground and sieved using 1mm sieve (AOAC, 1980). The composite sample in each year was taken to laboratory and analyzed for the determination of physical and chemical properties (Table 3). Total nitrogen, as determined by macro-Kjeldahl extraction (Bremner, 1965). Available phosphorus was determined by Bray 1 method (Bray and Kurz, 1945). Exchangeable cations were determined from ammonium acetate leachate (Black, 1965), using atomic absorption spectrophotometry for calcium (Ca) and magnesium (Mg), and flame photometry for sodium (Na) and potassium (K).

The land was double harrowed and then ridged 75 cm apart. Plots were demarcated after ridging with well-formed borders between plots (1 m) and replications (1.5 m) to minimize nutrient seepage, that is, flow from one position to the other.

Sowing was done by hand on 11th and 9th July in 2006, 2007 respectively and 17th June in 2008 after a good rain to provide moisture for good germination. Two seeds were planted per hole at the spacing of 25 cm between holes, and the seedlings were later thinned to one plant per stand at two weeks after sowing. This gave plant population of 53,333 plants per hectare.

The nutrients applied were N, P, K and S where by P, K, S and 75% of N were applied at 3 weeks after sowing while the remaining 25% of N at 6 weeks after sowing. P and K were equally applied to all plots at the rate of 26 and 50 kg/ha respectively, while N and S were varied according to the rates used for the trial (0, 60, 120 and 180 kg N $ha^{-1}$ and 0, 5, 10 and 15 kg S $ha^{-1}$). Nitrogen for each rate was applied in two doses of 75 and 25%. First dose of N was applied at 3 weeks after sowing along with the whole of P, K and S, while the second dose was applied at 6 weeks after sowing at the time of remoulding, that is, earthen up or putting soil to the base of the crop.

Weeding was done manually using hoe to control weeds at 3 and 6 weeks after sowing. Second weeding was followed by second dose of N fertilizer application and remoulding to cover the applied N and give support to the crop against lodging. Chemical weed control was not applied during this trial.

Stem borer infestation was observed at 3 weeks after sowing, which was controlled by spraying with a combination of cypermethrin and dimethoate at the rate of 0.03 and 0.25 kg active ingredient per hectare respectively. No disease was observed throughout the period of the trial.

Ear and flag leaves from five plants in each plot were collected at 50% tasselling, oven dried and ground into powder using electrical grinder. Grains were also randomly selected from each plot sun dried and ground into powder using electrical grinder. The powder was then sieved using 1 mm-mesh sieve and one gram was used for laboratory analysis for N and S. One gram of the sieved sample was digested using sulfuric acid and perchloric acid with copper and sodium sulfate acting as catalysts. The digest was then used to determine N and S content of the leaves and grains. Part of the digest was distilled into boric acid and the distillate was then titrated against a standard hydrochloric acid (HCl) and the percent N and S contents were determined from the titre using Macro – Kjedhal (Bremner, 1965; IITA, 1975).

Data collected were subjected to statistical analysis of variance and means of treatments were compared using Duncan Multiple Range Test (DMRT) (Duncan, 1955).

## RESULT

### Ear leaf N (g/kg) 2006

Interaction between nitrogen and sulfur significantly influenced uptake of N by quality protein maize in 2006 (Table 1). Interaction between 120 kg N/ha and 15 kg S/ha influenced higher N uptake but statistically similar with all the 4 N rates and 5 kg S/ha and also 0 kg N/ha and 15 kg S/ha. Lowest N uptake was observed when 60 kg N/ha interacts with 15 kg S/ha though at par with interaction between 0 and 60 kg N/ha and 0, 10 kg S/ha, likewise between 180 kg N/ha and 0, 15 kg S/ha.

### Ear leaf N (g/kg) 2007

Nitrogen uptake was also significantly influenced by interaction between nitrogen and sulfur in 2007. When 0 kg N/ha interacts with 5, 10, and 15 kg S/ha a higher N uptake by QPM was observed, but statistically similar with influence of interaction between 60 kg N/ha and 5 and 15 kg S/ha then between 120 kg N/ha and 10 kg S/ha and also between 180 kg N/ha and 10 and 15 kg S/ha. Lowest N uptake was given by the interaction between 120 kg N/ha and 15 kg S/ha even though at par with interaction between 0 kg N/ha and 0 kg S/ha; 60 kg N/ha and 10 kg S/ha; 120 kg N/ha and 5 kg S/ha; 180 kg N/ha and 0 and 5 kg S/ha.

### Ear leaf N (g/kg) 2008

When nitrogen uptake was observed in 2008, interaction between 0 kg N/ha and 5 kg S/ha and that between 120 kg N/ha and 0 kg S/ha produced similar but significantly higher N uptake than the other interactions. Interaction between 0 kg N/ha and 0, 10 and 15 kg S/ha produced similar but statistically lower N uptake than its interaction with 5 kg S/ha. When 60 kg N/ha interacts with 10 kg S/ha the uptake of N was significantly higher than when it interacts with 0, 5 and 15 kg S/ha. Similarly the interaction between 120 kg N/ha and 0 kg S/ha influenced significantly higher N uptake than its interaction with 5, 10 and 15 kg S/ha. A significantly higher N was absorbed by quality protein maize (QPM) when 180 kg N/ha interacts with 15 kg S/ha compared with the N uptake influenced by its interaction with 0, 5 and 10 kg S/ha which produced statistically similar N uptake.

### Flag leaf N (g/kg) 2007

Interaction between 0 and 180 kg N/ha with 15 kg S/ha produced significantly higher N uptake than their interaction with 0, 5 and 10 kg S/ha which gave statistically similar N uptake. The influence exerted by the interaction between 120 kg N/ha and 0 and 15 kg S/ha were similar and statistically higher than its interaction with 5 and 10 kg S/ha which were also statistically similar. However, when 180 kg N/ha interacts with 15 kg S/ha a significantly higher N uptake was observed than when it interacts with 0, 5 and 10 kg S/ha for which the influence on N uptake was similar.

### Flag leaf N (g/kg) 2008

Interaction between 120 kg N/ha and 10 kg S/ha influenced higher N uptake but statistically similar with interaction between all the N rates (0, 60, 120 and 180 kg N/ha) and 0 kg S/ha; 60, 120 and 180 kg N/ha and 5 and 15 kg S/ha; 0 and 60 kg N/ha and 10 kg S/ha. Similar and significantly lower N uptake was observed when 0 kg N/ha interacts with 5 and 15 kg S/ha.

### Maize grain analysis after harvest 2006 N (g/kg)

Uptake of N by quality protein maize in 2006 was influenced by nitrogen and sulfur interaction. This was observed through nitrogen content in the grain analysis. A significantly higher N uptake was influenced by the interaction between 120 kg N/ha and 5 kg S/ha. Interaction between 0 kg N/ha and 0 and 10 kg S/ha produced similar and significantly lower N uptake than its interaction with 5 and 15 kg S/ha. Interaction between 60

**Table 1.** Effects of Nitrogenand Sulfurfertilizer Interaction on up-take of N by QPM.

| Treatment | 0 kg S/ha | 5 kg S/ha | 10 kg S/ha | 15 kg S/ha |
|---|---|---|---|---|
| | Ear leaf N (g/kg) 2006 | | | |
| 0 kg N/ha | $15.325^{efg}$ | $17.5^{abc}$ | $15.525^{d-g}$ | $16.85^{a-e}$ |
| 60 kg N/ha | $15.775^{c-g}$ | $17.075^{a-e}$ | $14.65^{fg}$ | $14.45^{g}$ |
| 120 kg N/ha | $16.625^{b-e}$ | $16.85^{a-e}$ | $16.425^{c-f}$ | $18.6^{a}$ |
| 180 kg N/ha | $14.7^{fg}$ | $18.4^{ab}$ | $17.275^{a-d}$ | $15.55^{d-g}$ |
| S.E. ± | 0.5680 | | | |
| | Ear leaf N (g/kg) 2007 | | | |
| 0 kg N/ha | $20.8^{c-f}$ | $25.875^{a}$ | $24.5^{ab}$ | $23.2^{a-d}$ |
| 60 kg N/ha | $22.3^{b-e}$ | $22.775^{a-d}$ | $18.75^{f}$ | $22.775^{a-e}$ |
| 120 kg N/ha | $22.3^{b-e}$ | $21.025^{c-f}$ | $23.85^{abc}$ | $18.15^{f}$ |
| 180 kg N/ha | $19.075^{ef}$ | $20.35d^{ef}$ | $23.4^{a-d}$ | $23.85^{abc}$ |
| S.E. ± | 1.0296 | | | |
| | Ear leaf N (g/kg) 2008 | | | |
| 0 kg N/ha | $17.385^{b-e}$ | $22.1325^{a}$ | $15.3325^{ef}$ | $15.2^{ef}$ |
| 60 kg N/ha | $16.4525^{c-f}$ | $13.7925^{f}$ | $18.1325^{bcd}$ | $16.1275^{c-f}$ |
| 120 kg N/ha | $23.83^{a}$ | $18.8875^{bc}$ | $17.7175^{b-e}$ | $19.285^{b}$ |
| 180 kg N/ha | $15.3825^{def}$ | $15.325^{ef}$ | $15.065^{ef}$ | $18.885^{bc}$ |
| S.E. ± | 0.8550 | | | |
| | Flag leaf N (g/kg) 2007 | | | |
| 0 kg N/ha | $21.675^{ef}$ | $23.85^{b-e}$ | $22.1d^{ef}$ | $25.15^{a-d}$ |
| 60 kg N/ha | $25.375^{abc}$ | $22.75^{c-f}$ | $27.375^{a}$ | $26.5^{ab}$ |
| 120 kg N/ha | $25.4^{abc}$ | $21.225^{ef}$ | $20.125^{f}$ | $25.825^{abc}$ |
| 180 kg N/ha | $20.575^{f}$ | $22.775^{c-f}$ | $22.1^{def}$ | $25.375^{abc}$ |
| S.E. ± | 0.9821 | | | |
| | Flag leaf N (g/kg) 2008 | | | |
| 0 kg N/ha | $21.0175^{ab}$ | $16.64^{cd}$ | $21.15^{ab}$ | $16.0425^{d}$ |
| 60 kg N/ha | $21.105^{ab}$ | $20.4025^{ab}$ | $21.5925^{ab}$ | $20.67^{ab}$ |
| 120 kg N/ha | $20.43^{ab}$ | $22.765^{ab}$ | $23.25^{a}$ | $23.0325^{a}$ |
| 180 kg N/ha | $22.15a^{b}$ | $19.2325^{bc}$ | $19.2875^{bc}$ | $22.935^{a}$ |
| S.E. ± | 1.0179 | | | |
| | Maize grain analysis after harvest 2006 N (g/kg) | | | |
| 0 kg N/ha | $11.325^{cd}$ | $12.675^{b}$ | $11.075^{d}$ | $12.6^{b}$ |
| 60 kg N/ha | $12.55^{b}$ | $12.05^{bcd}$ | $12.625^{b}$ | $11.725^{bcd}$ |
| 120 kg N/ha | $11.975^{bcd}$ | $14.05^{a}$ | $12.25^{bcd}$ | $12.4^{bc}$ |
| 180 kg N/ha | $12.05^{bcd}$ | $12.375^{bc}$ | $12.35^{bc}$ | $12.125^{bcd}$ |
| S.E. ± | 0.35941 | | | |
| | Maize grain analysis after harvest 2008 N (g/kg) | | | |
| 0 kg N/ha | $15.15^{ab}$ | $14.335^{a-e}$ | $13.735^{b-e}$ | $13.7825^{b-e}$ |
| 60 kg N/ha | $13.45^{b-e}$ | $13.235^{b-e}$ | $12.965^{cde}$ | $16.1375^{a}$ |
| 120 kg N/ha | $13.815^{b-e}$ | $14.1775^{a-e}$ | $14.52^{a-d}$ | $12.365^{e}$ |
| 180 kg N/ha | $14.6025^{a-d}$ | $13.9225^{b-e}$ | $12.9325^{de}$ | $15.1075^{abc}$ |
| S.E. ± | 0.6326 | | | |

Means followed by the same letter(s) within a treatment group are not significantly different at 5% level of significance using dMRT.

and 180 kg N/ha and all the S rates (0, 5, 10 and 15 kg S/ha) did not differ in their influence on N uptake.

### Maize grain analysis after harvest 2008 N (g/kg)

Maize grain analysis in 2008 indicates that N uptake by quality protein maize was not significantly different when the influence of the interaction between all rates of nitrogen (0, 60, 120, and 180 kg N/ha) and 0, 5 and 10 kg S/ha. Variation in the uptake of N was observed when the different nitrogen rates interacted with 15 kg S/ha. Higher N uptake was given by the interaction between 60 kg N/ha and 15 kg S/ha, though at par with N uptake influenced by the interaction between 180 kg N/ha and 15 kg S/ha.

### Ear leaf S (mg/kg) 2006

Higher S uptake was observed from the interaction

**Table 2.** Effects of Nitrogen and Sulfur fertilizer interaction on up-take of Sby QPM.

| Treatment | 0 kg S/ha | 5 kg S/ha | 10 kg S/ha | 15 kg S/ha |
|---|---|---|---|---|
| | **Ear leaves S (mg/kg) 2006** | | | |
| 0 kg N/ha | 475[bcd] | 270[c-f] | 365[c-f] | 315[c-f] |
| 60 kg N/ha | 347.5[c-f] | 410[b-f] | 202.5[ef] | 150[f] |
| 120 kg N/ha | 525[bc] | 512.5[bcd] | 287.5[c-f] | 407.5[c-f] |
| 180 kg N/ha | 677.5[ab] | 825[a] | 245[def] | 467.5[b-e] |
| S.E. ± | 80.8132 | | | |
| | **Ear leaf S (mg/kg) 2007** | | | |
| 0 kg N/ha | 317.015[b] | 422.6325[a] | 264.145[bc] | 430.2[a] |
| 60 kg N/ha | 184.91[cde] | 169.165[de] | 263.95[bc] | 154.7425[e] |
| 120 kg N/ha | 211.325[cde] | 233.9575[b-e] | 256.6075[bcd] | 252.9475[bcd] |
| 180 kg N/ha | 233.9575[b-e] | 249.0625[bcd] | 207.57[cde] | 177.375[cde] |
| S.E. ± | 27.501 | | | |
| | **Ear leaf S (mg/kg) 2008** | | | |
| 0 kg N/ha | 12150[gh] | 11875[h] | 12375[fgh] | 11725[h] |
| 60 kg N/ha | 14050[b-e] | 13725[cde] | 14450[abc] | 14875[ab] |
| 120 kg N/ha | 13350[def] | 13925[b-e] | 14225[a-d] | 15100[a] |
| 180 kg N/ha | 13450[cde] | 13575[cde] | 13125[efg] | 14900[ab] |
| S.E. ± | 317.47 | | | |
| | **Flag leaf S (mg/kg) 2008** | | | |
| 0 kg N/ha | 18000[a-d] | 17475[cd] | 17350[d] | 18000[a-d] |
| 60 kg N/ha | 18250[a-d] | 17650[bcd] | 18500[abc] | 17675[bcd] |
| 120 kg N/ha | 18700[ab] | 18875[a] | 17500[cd] | 18350[a-d] |
| 180 kg N/ha | 17350[d] | 17725[bcd] | 18800[a] | 18150[a-d] |
| S.E. ± | 314.99 | | | |
| | **Maize grain analysis after harvest 2008 S (mg/kg)** | | | |
| 0 kg N/ha | 1400[abc] | 1500[ab] | 1125[bc] | 1375[abc] |
| 60 kg N/ha | 1250[bc] | 1225[bc] | 1175[bc] | 1475[ab] |
| 120 kg N/ha | 1050[c] | 1350[abc] | 1400[abc] | 1025[c] |
| 180 kg N/ha | 1350[abc] | 1400[abc] | 1100[bc] | 1675[a] |
| S.E. ± | 122.31 | | | |
| | **Maize grain analysis after harvest S (mg/kg) 2006** | | | |
| 0 kg N/ha | 925[b-e] | 975[bcd] | 1050[abc] | 950[bcd] |
| 60 kg N/ha | 875[cde] | 975[bcd] | 950[bcd] | 1075[ab] |
| 120 kg N/ha | 1075[ab] | 825[de] | 1175[a] | 900[b-e] |
| 180 kg N/ha | 875[cde] | 850[de] | 650[f] | 750[e] |
| S.E. ± | 53.805 | | | |

Means followed by the same letter(s) within a treatment group are not significantly different at 5% level of significance using dMRT.

between 180 kg N/ha and 5 kg S/ha but statistically similar with S uptake influenced by interaction between 180 kg N/ha and 0 kg S/ha (Table 2). Interaction between each of 0, 60 and 120 kg N/ha and all the S rates did not show any significant difference on uptake of S in the year 2006.

## Ear leaf S (mg/kg) 2007

Interaction between 0 kg N/ha and 15 kg S/ha produced higher S uptake than the other interactions though statistically similar when it interacts with 5 kg S/ha. Similar but lower S uptake was observed from the influence of the interaction between 60 kg N/ha and 0, 5 and 15 kg S/ha compared to its interaction with 10 kg S/ha. Interaction between 120 and 180 kg N/ha and all the S rates are not significantly different.

## Ear leaf S (mg/kg) 2008

Highest S uptake was influenced by the interaction between 120 kg N/ha and 0 kg S/ha, though at par with

**Table 3.** Physical and chemical properties of the soils of the experimental sites at Samaru Zaria in 2006, 2007 and 2008.

| Soil characteristic | Soil Depth (0 – 30 cm) | | |
|---|---|---|---|
| | **2006** | **2007** | **2008** |
| **Physical Characteristic (%)** | | | |
| Sand | 36.40 | 51.40 | 31.40 |
| Silt | 50.00 | 37.50 | 50.00 |
| Clay | 13.60 | 11.10 | 18.60 |
| Textural Class | Loam | Sandy Loam | Loam |
| **Chemical characteristics** | | | |
| pH 1:2.5 in $H_2O$ | 5.91 | 6.44 | 6.01 |
| pH 1:2.5 in $CaCl_2$ | 5.69 | 5.15 | 5.28 |
| Organic Carbon (%) | 1.44 | 0.59 | 0.90 |
| Total Nitrogen (%) | 0.27 | 0.11 | 0.18 |
| Available Phosphorus (mg/kg) | 22.40 | 7.35 | 23.45 |
| **Exchangeable Bases (Cmol/kg)** | | | |
| Ca | 4.80 | 3.00 | 3.60 |
| Mg | 3.00 | 1.80 | 3.00 |
| K | 0.28 | 0.41 | 0.33 |
| Na | 0.61 | 0.87 | 0.83 |
| S | 5.50 | 7.50 | 8.00 |
| CEC | 8.69 | 6.08 | 7.76 |
| ECEC | 9.29 | 6.18 | 8.16 |
| Total Acidity | 0.60 | 0.10 | 0.20 |

uptake influenced by interaction between 0 kg N/ha and 5 kg S/ha. Interaction between 0 kg N /ha and 0, 10 and 15 kg S/ha gave similar and significantly lower S uptake compared to its interaction with 5 kg S/ha. Interaction between 60 kg N/ha and 0, 5 and 15 kg S/ha produced similar but statistically lower S uptake than its interaction with 10 kg S/ha. When 120 kg N/ha interacts with 0 kg S/ha, a significantly higher S uptake was observed compared to its interaction with the other three S rates which produced statistically S uptake. The absorption of S by maize plant was similar when 180 kg N/ha interacts with 0, 5 and 10 kg S/ha as against its interaction with 15 kg S/ha.

## Flag leaf S (mg/kg) 2008

Interaction between each of 0 and 60 kg N/ha and all the S rates did not statistically differ in their influence on S uptake by QPM in the year 2008. Lower S uptake was observed when 120 kg N/ha interacts with 10 kg S/ha compared to its interaction with 0, 5 and 15 kg S/ha which were statistically similar. Similar uptake was also shown by the interaction between 180 kg N/ha and 0, 5 and 15 kg S/ha compared to its interaction with 10 kg S/ha.

## Maize grain analysis after harvest 2006 S (mg/kg)

Maize grain analysis in 2006 showed that sulfur uptake by QPM was affected by interaction between nitrogen and sulfur. When 0 kg N/ha interacts with all the S rates there was no significant difference in S uptake. However, the interaction between 60 kg N/ha and 0, 5 and 10 kg S/ha produced similar and statistically lower S uptake than when it interacts with 15 kg S/ha. Interaction between 120 kg N/ha and 0 and 10 kg S/ha influenced higher S uptake than its interaction with 5 and 15 kg S/ha. Interaction between 180 kg S/ha and 10 kg S/ha significantly influenced lower S uptake compared to its interaction with 0, 5 and 15 kg S/ha which were statistically similar.

## Maize grain analysis after harvest 2008 S (mg/kg)

Interaction between each of 0, 60 and 120 kg N/ha and all the S rates produced statistically similar S uptake. Interaction between 180 kg N/ha and 0, 5 and 10 kg S/ha produced similar and lower S uptake than its interaction with 15 kg S/ha.

## DISCUSSION

Interaction between nitrogen and sulfur significantly influenced some growth, yield and yield components. This was probably because both nitrogen and sulfur are important nutrients for growth and development which led to higher photosynthetic activities that resulted in the production of enough assimilate for subsequent translocation for higher yield. Sulfur and nitrogen relationships were established in terms of dry matter and yield in several crops in many studies (Zhao et al., 1993; McGrath and Zhao, 1996; Ahmad et al., 1998; Jamal et al., 2005; 2006a; 2010)

The utilization of nitrogen depends in a high degree on the balancing of nitrogen dose with the dose of sulfur (Grzebisz and Gaj, 2007, Seidler and Mamzer, 1994; Wyszkowski, 2000, 2001). Similar finding was reported by Muhammad et al. (2004) that grain number per ear was significantly affected by the interaction between N and S. Maize crop fertilized at the rate of 150 and 20 kg N and S/ha respectively produced significantly maximum grain number per ear (271.7) but minimum grain number per ear was recorded in case of control plot (Muhammad et al., 2004).

It was also observed that N and S interaction significantly influenced plant tissue N and S content. This observation agreed with that of Ray and Mughogho (2000) who reported that N and S content of plant leaf increased with application of the two nutrients than when only one was applied especially in nutrient deficient areas. Fazli et al. (2008) found that uptake of N was considerably reduced under S deficiency. A number of studies indicated synergistic effect of combined application of S and N on the uptake of these nutrients by maize and rapeseed (Fazli et al., 2008).

Sulfur addition, however, significantly increased the percent N in grain. Sulfur is an important nutrient for plant growth and development. Sulfur interactions with nitrogen are directly related to the alteration of physiological and biochemical responses of crops, and thus required to be studied in depth. This would help to understand nutritional behavior of sulfur in relation to nitrogen nutrients and provide guidelines for inventing balanced fertilizer recommendations in order to optimize yield and quality of crops (Fazli et al., 2008; Jamal et al., 2010).

## Conflict of Interest

The authors have not declared any conflict of interest.

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academicJournals

Vol. 10(28), pp. 2695-2704, 9 July, 2015
DOI: 10.5897/AJAR2015.9902
Article Number: 602EC1F53960
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

*Full Length Research Paper*

# Effect of silicon fertilization on mineral composition of rice under *Typic Ustochrept* soils

**J. K. Malav[1]*, K. C. Patel[2] and M. Sajid[1]**

[1]College of Agriculture, Anand Agricultural University, Jabugam–391155, Ta- Bodeli Dist. Chota-udaipur Gujarat, India.
[2]Department of Agricultural Chemistry and Soil Science, B. A. College of Agriculture, AAU, Anand, Gujarat, India.

Received 12 May, 2015; Accepted 3 June, 2015

**A pot experiment was conducted in 2012 in the greenhouse of the Anand Agricultural University Anand Gujarat, India. Treatments were arranged in a factorial completely randomized complete design with silicon factor at four levels (0, 100, 200 and 300 ppm) with three replicates. Indian improved and high - yielding variety Gurajari was used. The Si application up to 200 mg kg$^{-1}$ soil significantly increased grain and straw yields of rice over the control under low (< 25 mg Si kg$^{-1}$ soil) and medium (25 - 50 mg Si kg$^{-1}$ soil) category soils, while it was up to 100 mg Si kg$^{-1}$ soil in high category soils. The soil, grain and straw samples were analyzed for their Si, P, K, S and Na content at harvest. The Si content in grain ranged from 1.77 to 2.69% in untreated plots, whereas in treating plots, it ranged from 1.85 to 3.40%. The average value of Si content in straw was 7.78, 7.46 and 7.82% in low, medium and high category soils, respectively.**

**Key words:** Silicon, yield, mineral composition, content, rice.

## INTRODUCTION

Silicon (Si) is the second most abundant element in the earth's crust and soil. It has been considered to be quasi-essential element for plant growth (Epstein and Bloom, 2005). Rice is a known silicon accumulator (Takahashi et al., 1990) and the plant is benefiting from Si nutrition (Singh et al., 2005). Consequently, there is a definite need to consider Si as an essential minor element to increase sustained rice productivity (Sudhakar et al., 2006).

Rice is the staple food of about half of the world's population. It is cultivated in an area of 158 m ha$^{-1}$ with the production level of 472 million tons and productivity of 4.32 t ha$^{-1}$. Globally, 23% of the total calorie (35% in Asia and 31% in India) and 16% of the total protein comes from rice. Hence, 2004 AD is the "International Year of Rice" had a slogan as "Rice is Life". Rice cultivation possesses a formidable place from the beginning of Indian agriculture and it has always played a significant role in our food and civilization. In India, rice is grown in an area 44 million ha with the production level of 105.92 million tons and average productivity of 2393 kg ha$^{-l}$ during 2011-2012. The rice occupies an area of over 44 million hectares and in Gujarat, total area under rice cultivation was 8.36 lakh ha with total production of 17.9 lakh million tons and productivity of 2141 kg ha$^{-1}$ during 2011-2012.

*Corresponding author. E-mail: jugalmalav966@gmail.com, Tel: -9510658413

Silicon is a beneficial element for plant growth, especially for grasses, ferns, and horsetails. Most research on plant silicon has focused on the role of plant silicon as a beneficial element against herbivore and pest stress, as well as on its protective role against abiotic stressors including salinity and heavy metal pollution (Fauteux et al., 2005; Epstein, 2009). Recent studies revealed that in grasses, silicon uptake affects nutrient stoichiometry, cellulose, and phenol content in aboveground plant parts (Schaller et al., 2012a, b). However, little is currently known whether silicon uptake by plants affects mineralization or sequestration rates of litter through impacts on hardly degradable compounds and nutrient stoichiometry.

The species accumulates high amounts of silica in the shoots (as amorphous silica deposits or phytoliths) (Schaller et al., 2012b, 2013). In this study, *Pleurotus australis* was grown under both Si-rich and Si-poor conditions, resulting in plants differing in nutrient stoichiometry and very low in phenol and cellulose content. We tested whether macro or micro nutrient release/fixation of the leaf litter differed significantly between both sets of litter during aquatic litter decomposition. Furthermore, it was tested whether silicon availability during plant growth affects the decomposition process itself. It was the authors' hypothesis that higher availability of silicon to plants could increase the decomposition process due to an alteration of litter quality. In addition, we tested how invertebrate shredders, which have a key function in decomposition, affected the decomposition dynamics of the used litter. Our hypothesis is that the decay rate increases by the activity of the invertebrates.

Furthermore, the invertebrates may be deterred of feeding on leaf litter by the high amount of phytoliths in treatment Si, as described for folivores, for which high density of phytoliths in the food results in enhanced mandible wear (Massey and Hartley, 2009). Inverter at feeding neutralizes the effect of different litter quality on mass loss during microbial litter decomposition. The interaction of litter silica content and invertebrate feeding may be explained as follows: The faster decay of litter with invertebrate feeding is commonly known. Higher silica content may result in more biofilm on the litter, which is preferred by the invertebrates (see above). In contrast, the high amount of phytoliths in treatment Si+ may result in lower feeding activity (see above). Overall, litter decay was fastest in the presence of invertebrates.

In soil, most of the Si is held in the crystalline structure of sand, silt and clay particles. Upon weathering of soil silicate minerals, released Si into soil solution is taken up by plants in the form of silicic acid ($H_4SiO_4$) (Faure, 1991). There is a need to identify the nature and magnitude of the Si status of different rice eco-systems and thereby developing suitable Si management agenda for obtaining or sustaining rice yield potentials of improved rice cultivars. Most of them apply an anion to replace adsorbed Si and have been tested by determining the correlations between Si analyzed in the extract and crop yield. Not all of them were intended to extract the complete amount of plant available Si.

## MATERIALS AND METHODS

To know the available silicon status in different rice growing *goradu* soils, 60 surface soil samples were collected from the rice fields of Anand and Kheda districts. These soil samples were analyzed for available Si using NaOAc buffer extracts (Korndorfer et al., 1999) and categorized into low (< 25 mg $kg^{-1}$), medium (25 to 50 mg $kg^{-1}$) and high (>50 mg $kg^{-1}$) (Table 1).

Available silicon was extracted using NaOAc (pH- 4.0) extracts and silicon in the extracting solution was determined spectrophotometerically as suggested by Imaizumi and Yoshida (1958). The Si concentration of the digested plant samples was determined by spectrophotometric method given by (Ma and Tamai, 2002).

Available phosphorus, potassium and Sulphur was analyzed as per the standard procedures advised by Jackson (1973) and Williams and Steinberg (1959), respectively.

## RESULTS AND DISCUSSION

### Available silicon status

The textural class of soils under study was sandy clay loam to clay loam with average clay content of more than 30.0%. The CEC ranged from 15.0 to 23.0 c mol (p+) $kg^{-1}$ soil. The soils of rice fields were neutral to alkaline in reaction, soluble salts (EC) were low to high, the organic carbon status was low and available $P_2O_5$ status was low to high category and available $K_2O$ status was in medium to high category, while available S status was in low to medium (10 to 20 mg $kg^{-1}$) category (Table 2).

The NaOAc extractable available silicon content in rice soils of Anand district ranged from 30.58 to 78.73 mg $kg^{-1}$ with a mean value of 47.74 mg $kg^{-1}$, while in soils of Kheda district, it ranged from 18.38 to 44.06 mg $kg^{-1}$ with a mean value of 28.78 mg $kg^{-1}$ (Table 3). An overall available Si status in both the districts ranged from 18.38 to 78.73 mg $kg^{-1}$ with a mean of 41.42 mg $kg^{-1}$. This could be attributed to the depletion of available Si due to continuous rice cultivation, low solubility and/or slow dissolution kinetics of soil Si (Lindsay, 1979; Drees et al., 1989), high uptake of Si by rice crop to the extent of 250 kg $ha^{-1}$ for producing grain yield of 5 t $ha^{-1}$ (Savant et al., 1997), limited attempts by farmers to recycle Si through crop residues and/or lack of balanced fertilization (Savant et al., 1997). The variation among different soils in the available Si could be mainly attributed to the differences in cultivation practices followed, cropping system, organic carbon content of the soil, soil reaction, rainfall, materials, topography of the land, soil type and type and nature of the crop residues incorporated etc.

The results are in agreement with those of Nayar et al. (1982). They revealed that the available Si extracted by

**Table 1.** Initial soil properties of the bulk samples used in pot house study.

| S/N | Soils (S) | pH (1:2.5) | EC ($dSm^{-1}$) (1:2.5) | OC (g $kg^{-1}$) | Av. $P_2O_5$ (Kg $ha^{-1}$) | Av. $K_2O$ (Kg $ha^{-1}$) | Av. S (ppm) | Av. Si (ppm) |
|---|---|---|---|---|---|---|---|---|
| **Low category** | | | | | | | | |
| 1 | S1 | 7.52 | 0.20 | 3.8 | 50.9 | 461 | 10.0 | 18.4 |
| 2 | S2 | 7.53 | 0.53 | 3.8 | 70.6 | 514 | 13.9 | 18.3 |
| 3 | S3 | 8.14 | 0.33 | 4.6 | 19.3 | 421 | 7.20 | 19.0 |
| **Medium category** | | | | | | | | |
| 4 | S4 | 7.71 | 0.18 | 3.5 | 19.6 | 371 | 16.3 | 44.7 |
| 5 | S5 | 7.74 | 0.31 | 3.4 | 42.4 | 253 | 10.0 | 44.7 |
| 6 | S6 | 8.00 | 0.32 | 2.7 | 34.1 | 325 | 15.8 | 44.7 |
| **High category** | | | | | | | | |
| 7 | S7 | 8.09 | 0.24 | 2.2 | 18.5 | 245 | 16.4 | 58.8 |
| 8 | S8 | 7.25 | 0.34 | 3.3 | 65.7 | 341 | 14.5 | 58.8 |
| 9 | S9 | 7.57 | 0.19 | 1.4 | 39.1 | 490 | 21.8 | 58.8 |

Estimation of silicon in soil and plant.

**Table 2.** Soil chemical properties of rice soils of middle Gujarat.

| Variables | pH (1:2.5) | EC ($dSm^{-1}$) | Org. C (%) | Av. $P_2O_5$ (Kg $ha^{-1}$) | Av. $K_2O$ (Kg $ha^{-1}$) | Av. S (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|
| **Anand District (40 samples)** | | | | | | |
| Range | 7.25-8.15 | 0.17 - 0.89 | 0.14-0.56 | 14.0-91.6 | 171-570 | 6.2-23.0 |
| Mean | 7.77 | 0.33 | 0.36 | 44.9 | 350 | 16.5 |
| **Kheda District (20 samples)** | | | | | | |
| Range | 7.35-8.94 | 0.20-1.06 | 0.14-0.52 | 4.7-89.0 | 189-730 | 6.2-19.5 |
| Mean | 8.00 | 0.48 | 0.35 | 43.6 | 426 | 11.3 |
| **Overall (60 samples)** | | | | | | |
| Range | 7.25-8.94 | 0.17-1.06 | 0.14-0.56 | 4.72-91.6 | 171-730 | 6.2-23.0 |
| Mean | 7.8 | 0.38 | 0.36 | 44.5 | 376 | 14.8 |

different extracts in soils of Kerala ranged from 8 to 435 mg $kg^{-1}$. Similarly, the available Si in soils of Coimbtore (Tamilnadu) ranged from 29 to 80 mg $kg^{-1}$ (Subramanian and Gopalaswamy, 1991). Nayar et al. (1977) reported that the available Si extracted by NaOAc ranged from 8 to 278 mg $kg^{-1}$ in soils of Orissa. Gontijo (2000) observed that soil Si values decreased with increased content of the sand in the soil. A soil having high percentage of sand tends to show low Si contents due to their poor capacity to supply Si to plants.

## Response to Si application by rice

The results given in Table 4 indicated that the application of Si at all levels significantly increased the grain and straw yields over control in all categories of soils under study. Among the different soils studied, low and medium category soils have responded to the applied Si up to 200 mg $kg^{-1}$ soil in achieving higher grain and straw yields over the control. Whereas, soils under the high category have recorded higher grain and straw yields with the application of 100 mg $kg^{-1}$ soil and then showed a declining trend.

The rice growing under soils having low to medium available Si status responded to Si levels to a greater extent than the soils having higher levels of available Si. The maximum yield obtained due to the supplement of varied levels of Si in different soils ranged from 10.70 to 16. 84 g $pot^{-1}$ and yield increase ranged from 1.84 to 8.81 g $pot^{-1}$. This variation may be attributed to differences in native available Si and response to the added Si fertilizer.

**Table 3.** Available Si and cation status in rice soils of middle Gujarat.

| | Av. Si (mg kg$^{-1}$) | Exch. Ca (me 100 g soil$^{-1}$) | Exch. Mg (me 100 g soil$^{-1}$) | Av. Fe (mg kg$^{-1}$) | Av. Zn (mg kg$^{-1}$) |
|---|---|---|---|---|---|
| **Anand District (40 samples)** | | | | | |
| Range | 30.58-78.73 | 24.0-122.0 | 58.8-304.0 | 10.5-77.7 | 0.44-5.2 |
| Mean | 47.74 | 55.0 | 103.0 | 34.6 | 1.7 |
| **Kheda District (20 samples)** | | | | | |
| Range | 18.38-44.06 | 12.0-198.0 | 55.2-178 | 19.0- 87.8 | 0.56-4.7 |
| Mean | 28.78 | 67.0 | 107.2 | 44.8 | 1.8 |
| **Overall (60 samples)** | | | | | |
| Range | 18.38-78.73 | 12.0-198.0 | 55.2-303.0 | 10.5-87.8 | 0.44-5.2 |
| Mean | 41.42 | 58.9 | 104.1 | 38.0 | 1.7 |

**Table 4.** Effect of Si rates in rice grain and straw yield in different soils.

| Category | Soils (S) | Si levels (mg kg$^{-1}$ soil) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Grain Yield (g pot$^{-1}$) | | | | Straw Yield (g pot$^{-1}$) | | | |
| | | $Si_0$ | $Si_{100}$ | $Si_{200}$ | $Si_{300}$ | $Si_0$ | $Si_{100}$ | $Si_{200}$ | $Si_{300}$ |
| Low | $S_1$ | 8.03 | 14.13 | 16.84 | 12.38 | 20.48 | 23.37 | 27.30 | 25.77 |
| | $S_2$ | 7.80 | 14.18 | 16.49 | 13.81 | 22.87 | 25.03 | 28.13 | 23.97 |
| | $S_3$ | 10.03 | 14.50 | 15.86 | 13.83 | 18.93 | 23.10 | 26.07 | 24.57 |
| | Mean | 8.62 | 14.27 | 16.40 | 13.34 | 20.76 | 23.84 | 27.17 | 24.77 |
| Medium | $S_4$ | 8.45 | 9.87 | 14.86 | 13.40 | 20.39 | 21.52 | 24.81 | 22.43 |
| | $S_5$ | 8.23 | 11.27 | 12.90 | 10.65 | 20.79 | 22.83 | 23.72 | 23.74 |
| | $S_6$ | 8.00 | 10.30 | 11.93 | 9.90 | 21.64 | 23.10 | 26.03 | 24.23 |
| | Mean | 8.22 | 10.48 | 13.23 | 11.32 | 20.94 | 22.48 | 24.86 | 23.47 |
| High | $S_7$ | 8.10 | 11.33 | 8.83 | 7.83 | 17.40 | 18.90 | 18.97 | 16.73 |
| | $S_8$ | 8.86 | 10.70 | 9.22 | 9.05 | 18.68 | 23.09 | 19.93 | 19.37 |
| | $S_9$ | 6.53 | 11.80 | 10.83 | 7.77 | 17.31 | 24.49 | 19.90 | 18.33 |
| | Mean | 7.83 | 11.28 | 9.63 | 8.22 | 17.80 | 22.16 | 19.60 | 18.14 |
| C.D. Soils | | 0.80 | | | | 1.09 | | | |
| Silicon | | 0.54 | | | | 0.73 | | | |
| S × Si | | 1.61 | | | | 2.18 | | | |
| C.V. % | | 8.93 | | | | 6.05 | | | |

Similar results were also observed by Korndorfer et al. (2001) in soils of Florida. Increase in rice yield under flooded condition was noticed with Si fertilization in Sri Lanka (Takijima et al., 1970), Thailand (Takahashi et al., 1990), India (Singh et al., 2006) and Florida (Datnoff et al., 1992). Snyder et al. (1986) showed that application of calcium silicate increased the rice yields in histosols mainly due to the supply of available Si and not due to supply of other nutrients. The effect of Si on reducing diseases unquestionably contributed to increase yields, but Si has also been shown to increase yield in the absence of disease (Datnoff et al., 1992).

## Effect of Si fertilization on silicon and nutrient content in rice

### *Nutrient content*

The results presented in Table 5 revealed that Si content in grain was significantly increased under medium and high categories of soils as compared to low category soils. The highest Si content in grain (2.74%) was recorded in soils having high status of available Si, that is, high category soils. The Si content in grain ranged from 1.77 to 2.69% in untreated plots, whereas in treating

**Table 5.** Effect of Si application of silicon content in rice grain and straw in different soils of middle Gujarat.

| Category | Soils (S) | Grain and straw Si content (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Si levels (mg kg$^{-1}$ soil) | | | | | | | | Mean (Soil) | |
| | | $Si_0$ | | $Si_{100}$ | | $Si_{200}$ | | $Si_{300}$ | | Grain | Straw |
| | | Grain | Straw | Grain | Straw | Grain | Straw | Grain | Straw | | |
| Low | $S_1$ | 1.77 | 6.53 | 1.85 | 7.73 | 2.01 | 7.12 | 2.18 | 8.06 | 1.95 | 7.36 |
| | $S_2$ | 1.89 | 7.43 | 2.76 | 8.03 | 2.74 | 8.29 | 3.01 | 8.14 | 2.60 | 7.98 |
| | $S_3$ | 2.28 | 6.93 | 3.00 | 7.74 | 2.50 | 8.37 | 2.32 | 9.00 | 2.52 | 8.01 |
| | Mean | 1.98 | 6.97 | 2.54 | 7.84 | 2.42 | 7.93 | 2.50 | 8.40 | 2.36 | 7.78 |
| Medium | $S_4$ | 2.30 | 6.47 | 3.24 | 7.13 | 2.61 | 7.47 | 2.46 | 8.50 | 2.65 | 7.39 |
| | $S_5$ | 2.13 | 5.35 | 2.76 | 6.78 | 2.37 | 8.84 | 2.77 | 8.73 | 2.51 | 7.43 |
| | $S_6$ | 2.23 | 6.02 | 2.69 | 7.66 | 2.77 | 7.93 | 2.90 | 8.66 | 2.65 | 7.57 |
| | Mean | 2.22 | 5.94 | 2.90 | 7.19 | 2.58 | 8.08 | 2.71 | 8.63 | 2.60 | 7.46 |
| High | $S_7$ | 2.66 | 6.83 | 2.92 | 7.33 | 3.36 | 7.68 | 3.03 | 8.54 | 2.99 | 7.60 |
| | $S_8$ | 2.69 | 7.13 | 2.92 | 8.67 | 3.40 | 8.62 | 3.35 | 8.90 | 3.09 | 8.33 |
| | $S_9$ | 1.90 | 6.67 | 2.31 | 7.10 | 2.12 | 8.13 | 2.25 | 8.25 | 2.14 | 7.54 |
| | Mean | 2.42 | 6.88 | 2.71 | 7.70 | 2.96 | 8.14 | 2.88 | 8.56 | 2.74 | 7.82 |
| Overall | Range | 1.77-2.69 | 5.35-7.43 | 1.85-3.24 | 6.78-8.67 | 2.01-3.40 | 7.12-8.84 | 2.18-3.35 | 8.06-9.00 | | |
| | Mean (Si) | 2.21 | 6.60 | 2.72 | 7.58 | 2.65 | 8.05 | 2.70 | 8.53 | | |

| | Grain | | Straw | |
|---|---|---|---|---|
| | S. Em. ± | C.D. at 5% | S. Em. ± | C.D. at 5% |
| Soils (S) | 0.12 | 0.33 | 0.11 | 0.30 |
| Silicon (Si) | 0.08 | 0.22 | 0.02 | NS |
| S x Si | 0.24 | NS | 0.06 | 0.17 |
| C.V. % | 15.87 | | 14.44 | |

| | S. Em. ± | | C.D. at 5% | |
|---|---|---|---|---|
| | Grain | Straw | Grain | Straw |
| Soil category | 0.07 | 0.02 | 0.19 | NS |
| Si within soil category | 0.136 | 0.04 | NS | 0.10 |
| Si (Low) | 0.24 | 0.01 | NS | NS |
| Si (Medium) | 0.24 | 0.01 | NS | 0.04 |
| Si (High) | 0.24 | 0.02 | NS | NS |

plots, it ranged from 1.85 to 3.40%. The highest value of Si content in grain was observed at $Si_{100}$ level at 2.54 and 2.90% under low and medium category soils, respectively, but further increment in Si levels, it decreased in both categories. Also, the effect of Si application significantly increased Si content in grain at all the levels over control ($Si_0$). The average value of Si content in straw was 7.78, 7.46 and 7.82% in low, medium and high category soils, respectively. The significant critical difference in Si content of straw due to different Si levels was observed only under medium category soils. The Si content in straw was significantly increased at $Si_{100}$, $Si_{200}$ and $Si_{300}$ levels over control (5.94%). Nayar et al. (1982)

**Table 6.** Effect of Si application of phosphorus content in rice grain and straw in different soils of middle Gujarat.

| Category | Soils (S) | Grain and straw Phosphorus content (%) Si levels (mg kg$^{-1}$ soil) $Si_0$ Grain | $Si_0$ Straw | $Si_{100}$ Grain | $Si_{100}$ Straw | $Si_{200}$ Grain | $Si_{200}$ Straw | $Si_{300}$ Grain | $Si_{300}$ Straw | Mean (Soil) Grain | Mean (Soil) Straw |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Low | $S_1$ | 0.48 | 0.13 | 0.49 | 0.13 | 0.53 | 0.14 | 0.57 | 0.12 | 0.52 | 0.13 |
| | $S_2$ | 0.38 | 0.13 | 0.40 | 0.14 | 0.37 | 0.12 | 0.37 | 0.14 | 0.38 | 0.13 |
| | $S_3$ | 0.39 | 0.20 | 0.42 | 0.21 | 0.43 | 0.23 | 0.38 | 0.22 | 0.41 | 0.21 |
| | Mean | 0.42 | 0.15 | 0.44 | 0.16 | 0.44 | 0.16 | 0.44 | 0.16 | 0.43 | 0.16 |
| Medium | $S_4$ | 0.35 | 0.15 | 0.35 | 0.20 | 0.37 | 0.15 | 0.32 | 0.22 | 0.35 | 0.18 |
| | $S_5$ | 0.52 | 0.21 | 0.56 | 0.23 | 0.52 | 0.22 | 0.53 | 0.20 | 0.53 | 0.22 |
| | $S_6$ | 0.39 | 0.19 | 0.38 | 0.18 | 0.40 | 0.17 | 0.36 | 0.18 | 0.38 | 0.18 |
| | Mean | 0.42 | 0.18 | 0.43 | 0.21 | 0.43 | 0.18 | 0.41 | 0.20 | 0.42 | 0.19 |
| High | $S_7$ | 0.36 | 0.18 | 0.39 | 0.17 | 0.37 | 0.19 | 0.36 | 0.16 | 0.37 | 0.17 |
| | $S_8$ | 0.57 | 0.21 | 0.47 | 0.20 | 0.50 | 0.22 | 0.49 | 0.20 | 0.51 | 0.21 |
| | $S_9$ | 0.45 | 0.24 | 0.45 | 0.20 | 0.45 | 0.20 | 0.44 | 0.21 | 0.45 | 0.22 |
| | Mean | 0.46 | 0.21 | 0.44 | 0.19 | 0.44 | 0.20 | 0.43 | 0.19 | 0.44 | 0.20 |
| Overall | Range | 0.35-0.57 | 0.13-0.24 | 0.35-0.56 | 0.13-0.23 | 0.37-0.53 | 0.12-0.23 | 0.32-0.57 | 0.12-0.22 | | |
| | Mean (Si) | 0.43 | 0.18 | 0.43 | 0.19 | 0.44 | 0.18 | 0.43 | 0.18 | | |

| | Grain S. Em. ± | Grain C.D. at 5% | Straw S. Em. ± | Straw C.D.at 5% |
|---|---|---|---|---|
| Soils (S) | 0.004 | 0.01 | 0.003 | 0.010 |
| Silicon (Si) | 0.003 | 0.01 | 0.002 | NS |
| S x Si | 0.009 | 0.02 | 0.007 | 0.02 |
| C.V. % | 3.55 | | 6.53 | |

| | S. Em. ± Grain | S. Em. ± Straw | C.D. at 5% Grain | C.D. at 5% Straw |
|---|---|---|---|---|
| Soil category | 0.003 | 0.002 | 0.01 | 0.01 |
| Si within soil category | 0.009 | 0.004 | 0.02 | 0.01 |
| Si (Low) | 0.009 | 0.01 | 0.02 | 0.02 |
| Si (Medium) | 0.009 | 0.01 | 0.02 | 0.02 |
| Si (High) | 0.005 | 0.01 | 0.01 | 0.02 |

whole plant increased with the progress of growth and was also during vegetative growth and high after flowering stage. The silica absorption was slow during the initial growth stages, but increased with the onset of the reproductive growth period after flowering.

Several researchers also observed that the $SiO_2$ content of straw ranged from 4.4 to 19.6% with an average of 11.0% (Imaizumi and Yoshida, 1958), 7.5 to 9.0% (Wu and Lian, 1965). Nayar et al. (1977) had indicated that the $SiO_2$ content in harvested straw and grain was 7.13 and 2.67%, respectively.

Similar results were also reported by Takahashi et al. (1990) in soils of Sri Lanka, Singh et al. (2006) in soils of India and Snyder et al. (1986) and Korndorfer et al. (2001) in some soils of Florida.

The nutrients viz., P, K, S and Na content in grain and straw differed significantly by Si levels among soil categories (Tables 6 to 9). The highest P and K content reported that the silica content of

**Table 7.** Effect of Si application of phosphorus content in rice grain and straw in different soils of middle Gujarat.

| Category | Soils (S) | Grain and straw Potassium content (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Si levels (mg kg$^{-1}$ soil) | | | | | | | | Mean (Soil) | |
| | | $Si_0$ | | $Si_{100}$ | | $Si_{200}$ | | $Si_{300}$ | | | |
| | | Grain | Straw | Grain | Straw | Grain | Straw | Grain | Straw | Grain | Straw |
| Low | $S_1$ | 0.57 | 1.05 | 0.54 | 1.02 | 0.55 | 0.93 | 0.59 | 1.17 | 0.56 | 1.04 |
| | $S_2$ | 0.87 | 1.31 | 0.90 | 1.07 | 0.88 | 0.93 | 0.90 | 1.39 | 0.89 | 1.17 |
| | $S_3$ | 0.55 | 0.85 | 0.65 | 1.04 | 0.65 | 1.12 | 0.72 | 1.19 | 0.64 | 1.05 |
| | Mean | 0.66 | 1.07 | 0.70 | 1.04 | 0.69 | 0.99 | 0.74 | 1.25 | 0.70 | 1.09 |
| Medium | $S_4$ | 0.79 | 1.16 | 0.78 | 1.12 | 0.80 | 1.25 | 0.78 | 1.39 | 0.78 | 1.23 |
| | $S_5$ | 0.48 | 1.31 | 0.49 | 1.59 | 0.50 | 1.60 | 0.56 | 1.60 | 0.51 | 1.52 |
| | $S_6$ | 0.78 | 0.99 | 0.87 | 1.07 | 0.85 | 0.97 | 0.76 | 1.04 | 0.81 | 1.01 |
| | Mean | 0.68 | 1.15 | 0.71 | 1.26 | 0.72 | 1.27 | 0.70 | 1.34 | 0.70 | 1.25 |
| High | $S_7$ | 0.62 | 1.08 | 0.76 | 1.09 | 0.83 | 1.27 | 0.80 | 1.13 | 0.75 | 1.14 |
| | $S_8$ | 0.86 | 0.96 | 0.92 | 0.93 | 0.87 | 0.90 | 0.96 | 0.96 | 0.90 | 0.93 |
| | $S_9$ | 1.11 | 1.33 | 1.09 | 1.39 | 0.97 | 1.36 | 1.14 | 1.32 | 1.08 | 1.35 |
| | Mean | 0.86 | 1.12 | 0.92 | 1.13 | 0.89 | 1.17 | 0.97 | 1.14 | 0.91 | 1.14 |
| Overall | Range | 0.48-1.11 | 0.85-1.33 | 0.49-1.09 | 0.93-1.59 | 0.50-0.97 | 0.93-1.60 | 0.56-1.14 | 0.96-1.60 | | |
| | Mean (Si) | 0.74 | 1.11 | 0.78 | 1.14 | 0.76 | 1.14 | | 1.24 | | |

| | Grain | | Straw | |
|---|---|---|---|---|
| | S. Em. ± | C.D. at 5% | S. Em. ± | C.D. at 5% |
| Soils (S) | 0.006 | 0.02 | 0.012 | 0.03 |
| Silicon (Si) | 0.004 | 0.01 | 0.008 | 0.02 |
| S x Si | 0.012 | 0.03 | 0.024 | 0.07 |
| C.V. % | 2.66 | | 3.55 | |

| | S. Em. ± | | C.D. at 5% | |
|---|---|---|---|---|
| | Grain | Straw | Grain | Straw |
| Soil category | 0.003 | 0.007 | 0.01 | 0.02 |
| Si within soil category | 0.007 | 0.014 | 0.02 | 0.04 |
| Si (Low) | 0.012 | 0.024 | 0.03 | 0.07 |
| Si (Medium) | 0.012 | 0.024 | 0.03 | 0.07 |
| Si (High) | 0.012 | 0.024 | 0.03 | 0.07 |

the silicon.

The interaction effect of soil and Si levels was significant for P, K, S and Na contents of rice grain and straw. Similar results were also reported by Okuda and Takahashi (1962b) who stated that added silicon increased the translocation rate of absorbed phosphorous to the grain, especially at the phosphorus deficient level. This effect might have resulted from the action of silicon, in which the increasing amount of silicon decreased the iron content of rice plant. Si controls the chemical and biological properties of soil with the following benefits: Silicon reduced leaching of phosphorous (P) and potassium (K) (Sadgrove, 2006), reduced Aluminium (Al), Iron (Fe), Manganese (Mn) and

**Table 8.** Effect of Si application of sulfur content of rice grain and straw in different soils of middle Gujarat.

| Category | Soils (S) | Grain and straw Sulphur content (%) Si levels (mg $kg^{-1}$ soil) Si$_0$ Grain | Si$_0$ Straw | Si$_{100}$ Grain | Si$_{100}$ Straw | Si$_{200}$ Grain | Si$_{200}$ Straw | Si$_{300}$ Grain | Si$_{300}$ Straw | Mean (Soil) Grain | Mean (Soil) Straw |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Low | $S_1$ | 0.81 | 0.89 | 0.86 | 0.86 | 0.72 | 0.86 | 0.81 | 0.87 | 0.80 | 0.87 |
| | $S_2$ | 0.72 | 0.74 | 0.82 | 0.73 | 0.83 | 0.67 | 0.85 | 0.93 | 0.80 | 0.77 |
| | $S_3$ | 0.65 | 0.96 | 0.76 | 0.86 | 0.65 | 0.91 | 0.85 | 0.86 | 0.73 | 0.90 |
| | Mean | 0.73 | 0.86 | 0.81 | 0.82 | 0.73 | 0.81 | 0.83 | 0.88 | 0.78 | 0.84 |
| Medium | $S_4$ | 0.65 | 0.76 | 0.63 | 0.78 | 0.63 | 0.87 | 0.69 | 0.73 | 0.65 | 0.79 |
| | $S_5$ | 0.81 | 0.82 | 0.82 | 0.78 | 0.85 | 0.78 | 0.86 | 0.87 | 0.83 | 0.81 |
| | $S_6$ | 0.80 | 0.43 | 0.87 | 0.44 | 0.81 | 0.54 | 0.85 | 0.47 | 0.83 | 0.47 |
| | Mean | 0.75 | 0.67 | 0.77 | 0.67 | 0.76 | 0.73 | 0.80 | 0.69 | 0.77 | 0.69 |
| High | $S_7$ | 0.84 | 0.77 | 0.85 | 0.75 | 0.74 | 0.72 | 0.84 | 0.75 | 0.82 | 0.75 |
| | $S_8$ | 0.88 | 0.72 | 0.82 | 0.85 | 0.87 | 0.78 | 0.84 | 0.77 | 0.85 | 0.78 |
| | $S_9$ | 0.63 | 0.82 | 0.64 | 0.79 | 0.67 | 0.89 | 0.57 | 0.92 | 0.62 | 0.86 |
| | Mean | 0.78 | 0.77 | 0.77 | 0.80 | 0.76 | 0.80 | 0.75 | 0.82 | 0.77 | 0.80 |
| Overall | Range | 0.63-0.88 | 0.43-0.96 | 0.63-0.87 | 0.44-0.86 | 0.63-0.87 | 0.54-0.91 | 0.57-0.86 | 0.47-0.93 | | |
| | Mean (Si) | 0.75 | 0.77 | 0.78 | 0.76 | 0.75 | 0.78 | 0.79 | 0.80 | | |

| | Grain S. Em. ± | Grain C.D. at 5% | Straw S. Em. ± | Straw C.D. at 5% |
|---|---|---|---|---|
| Soils (S) | 0.007 | 0.02 | 0.010 | 0.03 |
| Silicon (Si) | 0.00 | 0.01 | 0.007 | 0.02 |
| S x Si | 0.01 | 0.04 | 0.020 | 0.06 |
| C.V. % | 3.02 | | 4.52 | |

| | S. Em. ± Grain | S. Em. ± Straw | C.D. at 5% Grain | C.D. at 5% Straw |
|---|---|---|---|---|
| Soil category | 0.004 | 0.006 | NS | 0.02 |
| Si within soil category | 0.01 | 0.012 | 0.02 | 0.03 |
| Si (Low) | 0.01 | 0.020 | 0.04 | 0.06 |
| Si (Medium) | 0.01 | 0.020 | 0.04 | 0.06 |
| Si (High) | 0.01 | 0.020 | 0.04 | 0.06 |

heavy metal mobility (Matichenkov and Calvert, 2002), improved microbial activity (Matichenkov and Calvert, 2002), increased stability of soil organic matter, improved soil texture (Sadgrove, 2006), improved water holding capacity (Sadgrove, 2006), increased stability against soil erosion (Sadgrove, 2006), and increased cationic exchange capacity (CEC) (Camberato, 2001). Form of Si in soil present $SiO_2$ and as various silicates minerals and form of Si in soil solution present silicic acid [Si (OH) 4]. Islam and Saha (1969) inferred that the application of silicon

**Table 9.** Effect of Si application of Sodium content of rice grain and straw in different soils of middle Gujarat.

| Category | Soils (S) | Grain and straw Sodium content (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Si levels (mg kg$^{-1}$ soil) | | | | | | | | Mean (Soil) | |
| | | $Si_0$ | | $Si_{100}$ | | $Si_{200}$ | | $Si_{300}$ | | | |
| | | Grain | Straw | Grain | Straw | Grain | Straw | Grain | Straw | Grain | Straw |
| Low | $S_1$ | 0.26 | 0.31 | 0.27 | 0.29 | 0.29 | 0.31 | 0.27 | 0.32 | 0.27 | 0.31 |
| | $S_2$ | 0.34 | 0.34 | 0.32 | 0.32 | 0.33 | 0.37 | 0.34 | 0.37 | 0.33 | 0.35 |
| | $S_3$ | 0.42 | 0.22 | 0.44 | 0.22 | 0.44 | 0.21 | 0.44 | 0.23 | 0.43 | 0.22 |
| | Mean | 0.34 | 0.29 | 0.34 | 0.27 | 0.35 | 0.30 | 0.35 | 0.31 | 0.35 | 0.29 |
| Medium | $S_4$ | 0.34 | 0.36 | 0.36 | 0.34 | 0.33 | 0.38 | 0.31 | 0.38 | 0.34 | 0.36 |
| | $S_5$ | 0.40 | 0.23 | 0.35 | 0.24 | 0.34 | 0.29 | 0.33 | 0.27 | 0.36 | 0.26 |
| | $S_6$ | 0.46 | 0.24 | 0.47 | 0.22 | 0.47 | 0.27 | 0.45 | 0.22 | 0.46 | 0.24 |
| | Mean | 0.40 | 0.28 | 0.39 | 0.27 | 0.38 | 0.31 | 0.36 | 0.29 | 0.38 | 0.29 |
| High | $S_7$ | 0.37 | 0.32 | 0.32 | 0.37 | 0.34 | 0.35 | 0.34 | 0.36 | 0.34 | 0.35 |
| | $S_8$ | 0.23 | 0.21 | 0.26 | 0.18 | 0.20 | 0.22 | 0.24 | 0.18 | 0.23 | 0.20 |
| | $S_9$ | 0.34 | 0.37 | 0.36 | 0.34 | 0.36 | 0.35 | 0.34 | 0.34 | 0.35 | 0.35 |
| | Mean | 0.31 | 0.30 | 0.31 | 0.30 | 0.30 | 0.31 | 0.31 | 0.29 | 0.31 | 0.30 |
| Overall | Range | 0.23-0.46 | 0.21-0.37 | 0.26-0.47 | 0.18-0.37 | 0.20-0.47 | 0.21-0.38 | 0.24-0.44 | 0.18-0.38 | | |
| | Mean (Si) | 0.35 | 0.29 | 0.35 | 0.28 | 0.34 | 0.31 | 0.34 | 0.29 | | |

| | Grain | | Straw | |
|---|---|---|---|---|
| | S. Em. ± | C.D. at 5% | S. Em. ± | C.D. at 5% |
| Soils (S) | 0.003 | 0.01 | 0.005 | 0.01 |
| Silicon (Si) | 0.002 | 0.01 | 0.003 | 0.01 |
| S x Si | 0.007 | 0.02 | 0.009 | 0.03 |
| C.V. % | 3.50 | | 5.61 | |

| | S. Em. ± | | C.D. at 5% | |
|---|---|---|---|---|
| | Grain | Straw | Grain | Straw |
| Soil category | 0.002 | 0.003 | 0.01 | 0.01 |
| Si within soil category | 0.004 | 0.005 | 0.01 | 0.02 |
| Si (Low) | 0.007 | 0.009 | NS | NS |
| Si (Medium) | 0.007 | 0.009 | 0.02 | 0.03 |
| Si (High) | 0.007 | 0.009 | 0.02 | 0.03 |

generally decreased the potassium content of rice plants.

## Conclusion

Among the different soils studied, low and medium category soils have responded to the applied Si up to 200 mg kg$^{-1}$ soil in achieving higher grain and straw yields over the control. Whereas, soils under the high category have recorded higher grain and straw yields with the application of 100 mg kg$^{-1}$ soil and then showed a declining trend. The application of Si at different rates recorded 1.85 to 3.40% Si content in the grain, whereas, it ranged from 1.77 to 2.69% under control. The highest Si content in grain (2.74%) was recorded in high category soils. The average value of Si content in straw was 7.78, 7.46 and 7.82% in low, medium and high category soils, respectively.

## Conflict of Interest

The authors have not declared any conflict of interest.

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academicJournals

Vol. 10(28), pp. 2705-2710, 9 July, 2015
DOI: 10.5897/AJAR2015.9911
Article Number: A26C2C353962
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

*Full Length Research Paper*

# Dry season bean production at different sowing dates under the South Minas Gerais conditions, Brazil

**Cristiane Fortes Gris[1*], Carlos Henrique Evaristo[1], Lucas Eduardo de Oliveira Aparecido[2], Denis Henrique Silva Nadaleti[1] and Willian César Freiria[1]**

[1]Instituto Federal do Sul de Minas Gerais, Campus Muzambinho, Brazil.
[2]Universidade Estadual Paulista, Departamento de Ciências Exatas, Jaboticabal, São Paulo, Brazil.

Received 12 May, 2015; Accepted 18 June, 2015

**The agro-climatic zoning classifies the southern region of Minas Gerais, Brazil, as being suitable for bean farming, especially in the dry season, where there are appropriate climatic conditions for growing. In this area, determining the optimum sowing dates is controlled by the climatic conditions and the adaptability of different cultivars. Therefore, the aim of this study was to evaluate the agronomic characteristics of some common bean cultivars submitted to different seeding times in the dry season in the South of Minas Gerais. The beans cultivars were used (Perola, TAA Bola Cheia and IAPAR 81) submitted to four sowing date (January 18, February 26, March 08 and March 20). The experimental was arranged in a randomized complete block design (RBD) with three replications in a factorial 4 × 3, corresponding to the four sowing dates and three bean cultivars and results were submitted to analysis of variance by the F test, and when there was a significant, means were compared by Tukey test at 5% and regression analysis. The water deficit was the weather element most influencing bean yield. There was interaction between cultivars and seeding times only on bean yield. The first sowing (January 18) provides the highest yield (30% more in relation the other) and the best agronomic characteristics, regardless of the cultivar. Cultivars Bola Cheia and Perola are the most productive.**

**Key words:** Phaseolus vulgaris, crop yield, water stress, main components.

## INTRODUCTION

Brazil is the largest producer of common bean (*Phaseolus vulgaris* L.) with a production of 3.34 million tons in 2014 in all country (Companhia Nacional de Abastecimento, 2014/15). The bean is grown almost all Brazilian States, in various environmental conditions and in different seeding times (Carneiro, 2002). The state of Minas Gerais is the second largest bean producing, with 592,200 tones (Companhia Nacional de Abastecimento, 2014/15). The agro-climatic zoning of the state of Minas Gerais showed that state is apt for bean crop, especially during the dry season (BRASIL, 2014).

The determination of sowing periods takes into account the key climate risk factors, seeking a lower probability of loss of crops (Aparecido et al., 2014a). Thus, the producers get better yields in a particular region, without providing increase production costs, especially in the dry season, which concentrates periods of water deficit at end of cycle (Wrege et al., 1999; Dalla Corte, 2003).

*Corresponding author. E-mail: cristiane.gris@muz.ifsuldeminas.edu.br

Producers are seeking periods without rain for planting. Thus, many producers seek to plant in the dry season, occurring in Brazil for the months of February to March, taking advantage of the end of the rainy season. This time, air temperatures are usually more appropriate, the problems with weeds and diseases are reduced (EMBRAPA, 2005).

The weather is the main factor that makes producers anticipates sowing dates of the dry season. However, the response to seeding periods is not connected only with seeding time, but also the cultivar (Wrege et al., 1999). Interaction of genotype with environment is a major problem for selection or appointment of cultivars of bean breeding program, with the alternative identification of cultivars with higher phenotypic stability and wide adaptability (Ribeiro et al., 2008).

Commons bean cultivated in different sowing dates when subjected to adverse weather demonstrated reductions in grain yield (Ramalho et al., 1998; Carbonell and Pompeu, 2000; Carbonell, 2001). Accordingly, preference should be given to cultivars with a wide adaptation to different cropping systems, mainly because of the seeding, defined by agroclimatic zoning of crop, most often early or delayed by farmers due to climate risks (Carbonell, 2001).

Few studies have been conducted to examine the optimum sowing date and cultivar selection for the different cultivated crops (Ribeiro, 2004; Aparecido et al., 2014b). Thus, the aim of this work was to evaluate the agronomic characteristics of some beans cultivars under different sowing dates in the dry season in the South of state of Minas Gerais, Brazil.

## MATERIALS AND METHODS

The experiment was conducted in Muzambinho, Minas Gerais, Brazil (21°22′33″S, 46°31′ 32″W, 1050 m of altitude), an important region of bean production. The classification of the predominant climate of the region is $B_4rB'_2a$ (humid with low water deficit) following Thornthwaite (1948) (Aparecido et al., 2015). The classification soil of the experimental area has rhodic Haplustox soil (EMBRAPA, 2006).

Data for maximum and minimum air temperature (°C) and rainfall (mm) were measured by Davis sensors. The sequential daily water balance was calculated as proposed by Thornthwaite and Mather (1955) using SYSWAB software (Gaspar et al., 2015). The potential evapotranspiration was estimated using the Thornthwaite (1948) method.

Meteorological data were used to calculate water deficiencies (DEF) (Equation 1), from the water balance calculation by the Thornthwaite and Mather (1955) method at monthly scale with available water capacity of 100 mm.

$$DEF = PET – AET \qquad (1)$$

where, DEF is water deficiency at the soil-plant system; PET is the potential evapotranspiration and AET is actual or real evapotranspiration

The selected cultivars were Perola, Bola Cheia and IAPAR 81. Perola has indeterminate growth habit, semi-erect, 90-day cycle with a mean duration of 46 days to flowering, white flower, with resistance to rust and mosaic-common (Yokoyama, 1999). The Bola Cheia has an indeterminate growth habit, semi-erect, intermediate cycle, 36 to 45 days to flowering, with white flower (SEPROTEC, 2014). And the cultivar IAPAR 81 has an indeterminate growth habit, erect, 92 days cycle, and mean of 42 days to flowering and white flower (IAPAR, 2014).

The experimental design was a randomized complete block design (RBD) with three replications in a factorial 4 × 3, with four seeding dates and three beans cultivars, totaling 36 installments. The experimental unit consisted of four rows with 5.0 m of width, spaced 0.50 m each one. The full experimental area had 20 m of size and 12 of wide.

Seeding of all cultivars was made on the following dates: January 18, February 26, March 08 and Marrch 20 of 2014. The gap between the first and second sowing dates was due to unusual dry spell that occurred in the region causing a delay for the second sowing day. The fertilization was applyed according to soil analysis, and the interpretations proposed by Barbosa and Gonzaga (2012), taking into account technology level 3. The authors used 300 kg $ha^{-1}$ formulated fertilizer 8-28-16, by applying 30 kg $ha^{-1}$ N, 25 days after emergence, using as N font ureia.

Data on soil analysis can be viewed below: pH – 6.0; P – 2.4 mg $dm^{-3}$; K - 81 mg $dm^{-3}$; Ca – 1.65 cmolc $dm^{-3}$; Mg – 0.84 cmolc $dm^{-3}$; Al – 0.03 cmolc $dm^{-3}$; H + Al – 2.57 cmolc $dm^{-3}$; Zn – 1.0 mg $dm^{-3}$; Fe – 32.3 mg $dm^{-3}$; Mn – 6.3 mg $dm^{-3}$; Cu – 1.2 mg $dm^{-3}$; B – 0.18 mg $dm^{-3}$; SB – 2.7 cmolc $dm^{-3}$; t – 2.7 cmolc $dm^{-3}$; T – 5.3 cmolc $dm^{-3}$, V – 51.2%; m – 1.1%; medium texture. Other agricultural practices were performed as recommended by the literature.

The harvest was performed manually in the developmental stage R9 (Gross and Kigel, 1994), where the following characteristics were evaluated: height: considering ground level until the last top node of the main stem and number of pods per plant, both obtained from 04 plants randomly in the useful portion; number of grains per pod: average score obtained in the pods of 04 plants randomly in the useful portion; grain yield (kg $ha^{-1}$), calculated in kg $ha^{-1}$ and mean weight of 100 seeds: determined from seeds harvested in the useful area of each plot, according to the methodology established by BRASIL (20014).

Yield was corrected to 13% moisture content, according to the equation:

$$PF = PI \times (100 – UI) / (100 – UF)$$

where, PF = corrected final weight of the sample; PI = initial weight of sample; UI = initial moisture content of the sample and UF = final moisture content of the sample (13%).

Results were analyzed using F test and the means were compared by Tukey test (at 5% significance) and regression analysis. "SISVAR" (Ferreira, 2011) program was used to perform all the statistical analysis.

## RESULTS AND DISCUSSION

The average temperature in the region during the study period ranged from 15.4 to 22.4°C (Figure 1). The water deficit (DEF) occurred from January to March with intensity of - 5 mm and May to July, reaching an intensity of - 6 mm (Figure 1). The values of air temperature were similar to the normal climatological conditions of 16 to 23ºC, but the values of DEF were not similar, because it typically does not occur, DEF in the period of January to March (INMET, 2015).

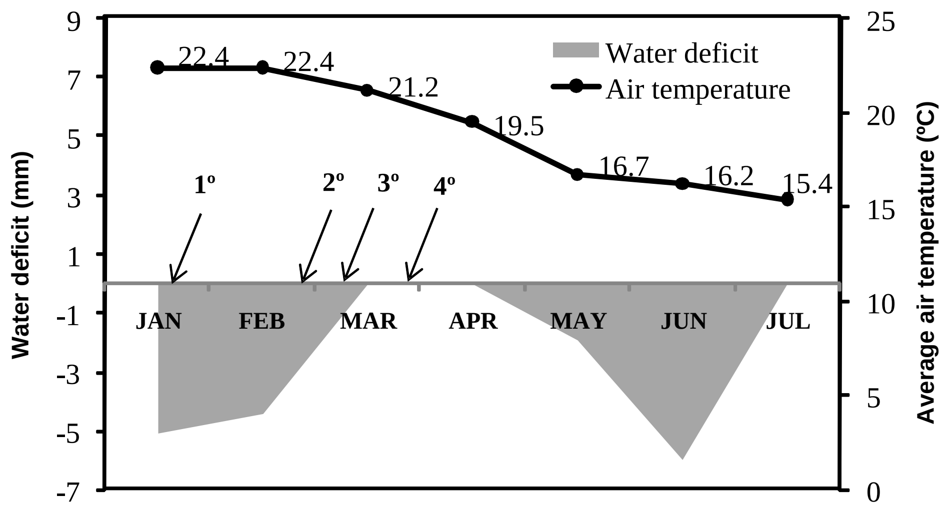


**Figure 1.** Water deficit (mm) and average air temperature (°C) in during the period from January to July of 2014 in the South of Minas Gerais – MG (1º; 2º; 3º and 4º is first, second, third and fourth sowing data, respectively).

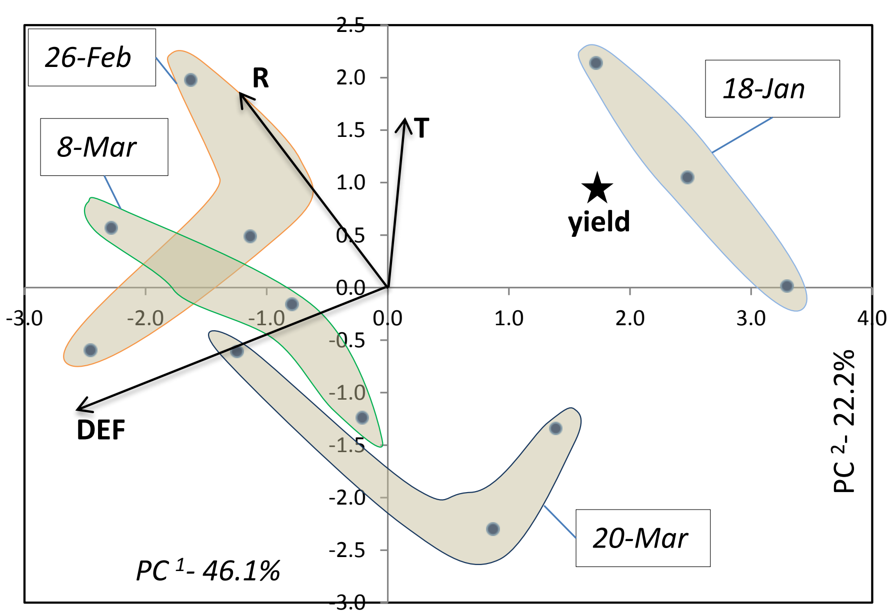


**Figure 2.** Biplot PC1 vs PC2 of dependent and independent variables and sowing dates (DEF = water deficit; T = Air temperature; R = rainfall).

The period of the first to second sowing dates there was a reduction in rainfall in the South of Minas (Figure 1), raining only 28% (68 mm) of normal (245 mm). The authors observed that in March there is a reduction of maximum and minimum temperatures in the region, and thus late bean plantations coincided with periods of minimum temperatures below the optimal range recommended for bean crop, that is between 18 to 24°C (EMBRAPA, 2005).

The development of the bean plants in the various sowing dates was dependent on the climatic conditions. The first two components ($PC_1$ and $PC_2$) of the PCA of all variables of growth and meteorological conditions together explained 68.3% of the total variability of the data. The development of the plants, mainly yield was most influenced by DEF (Figure 2), showing to be the most important variable climate (Aparecido et al., 2015).

There was a significant interaction between cultivars and sowing dates seeding only in the variable yield. The highest values of plant height (1.26 m), number of

**Table 1.** Results of plant height (PLH), number of pods per plant (NPP), number of seeds per pod (NSP), weight of 100 seeds (W100) and crop yield (Y) of bean of dry season in function of the sowing date in the South of Minas, MG.

| Sowing date | PLH (M) | NPP (unit) | NSP (unit) | W100 (g) | Y (kg ha$^{-1}$) |
|---|---|---|---|---|---|
| January 18 | 1.26$^{a}$ | 17.28$^{a}$ | 5.55$^{a}$ | 21.01$^{b}$ | 1.391$^{a}$ |
| February 26 | 0.82$^{b}$ | 8.69$^{b}$ | 4.75$^{b}$ | 25.99$^{a}$ | 963$^{c}$ |
| March 08 | 0.76$^{b}$ | 9.41$^{b}$ | 4.75$^{b}$ | 22.11$^{b}$ | 1.077$^{b}$ |
| March 20 | 0.84$^{b}$ | 10.11$^{b}$ | 5.31$^{ab}$ | 21.69$^{b}$ | 896$^{c}$ |
| CV (%) | 14.99 | 30.27 | 12.03 | 7.14 | 9.99 |

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

**Table 2.** Results of plant height (PLH), number of pods per plant (NPP), number of seeds per pod (NSP), weight of 100 seeds (W100) and crop yield (Y) of bean of dry season in the South of Minas, MG.

| Cultivars | PLH (M) | NPP (unit) | NSP (unit) | W100 (g) | Y (kg ha$^{-1}$) |
|---|---|---|---|---|---|
| Perola | 1.06$^{a}$ | 11.92$^{a}$ | 5.10$^{ab}$ | 24.55$^{a}$ | 1.136$^{a}$ |
| IAPAR 81 | 0.69$^{c}$ | 10.92$^{a}$ | 4.71$^{b}$ | 21.57$^{b}$ | 927$^{b}$ |
| Bolacheia | 0.92$^{b}$ | 11.29$^{a}$ | 5.46$^{a}$ | 21.98$^{b}$ | 1.181$^{a}$ |
| CV% | 14.99 | 30.27 | 12.03 | 7.14 | 9.99 |

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

pods per plant (17.28), number of grains per pod (5.55) and grain yield (1,391 kg ha$^{-1}$) were obtained from the seeding at January 18, regardless the used cultivar (Table 1).

The best plant height, number of pods per plant and yield were observed in the first sowing (Table 1). For seeds yield, plants seeded in January 18 produced 42.13% (412 kg ha$^{-1}$) more yield (978 kg ha$^{-1}$), possibly due to the higher response of yield components on this seeding. The highest 100-seed weight value of 25.9 was recorded by seeding in February 26, however, the grain yield was not affected by this increase. The highest yield obtained at study is close to the recorded for the dry season in Minas Gerais, according to data from Companhia Nacional de Abastecimento (2014/15), are around 1,387 kg ha$^{-1}$.

The number of pods per plant is the yield component most affected by water deficit, resulting a reduction in grain yield (Guimarães et al., 1996; Gomes et al., 2000), because the bean productivity is highly correlated with the yield components, number of pods per plant, number of grains per pod and seed weight (Costa and Zimmermann, 1988; Coimbra et al., 1999).

Perola showed the highest height values of plants and weight of 100 seeds. The highest yields were observed by Perola cultivars and Bola Cheia (Table 2). There were not differences among cultivars for the number of pods per plant, which showed mean values of 11.37 (average all cultivars) pods per plant. The Perola and Bola Cheia demonstrated similar responses to the agronomic characters.

The bean cultivars showed a quadratic response for grain yield (Figure 3). Cultivars Bola Cheia and Perola showed better performance when seed in the first sowing dates in mid-January, for the cultivar IAPAR 81 yield at the 3 firsts sowings were similar and higher than the last sowing date (March 20).

## Conclusion

The bean seeded in January 18 demonstrates higher yield, plant height number of pods per plant and number of seeds per pod in the regions of South of Minas Gerais. The authors observed that with the delay of sowing is the reduction of common bean yield.

The water deficit is the variable climate that most influences common bean yields in the South Minas. Cultivars Bola Cheia and Perola are more productive when compared with the cultivar IAPAR 81.

## Conflict of Interest

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

## ACKNOWLEDGEMENTS

The authors are grateful to Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais – IFSULDEMINAS for scholarship granted and financial support.

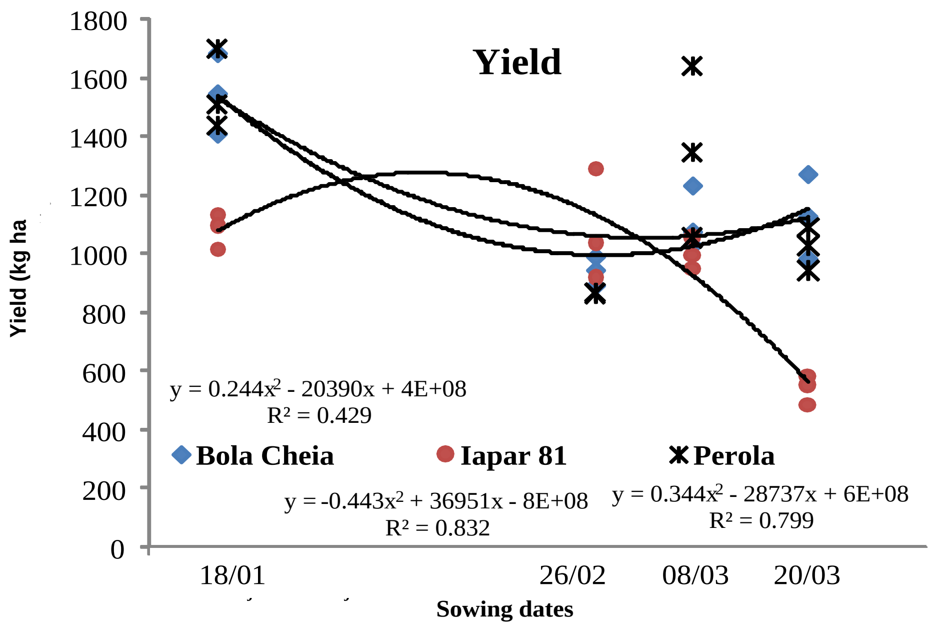


**Figure 3.** Yield (kg ha$^{-1}$) of the Bean in the dry season in function of the sowing dates in the South of Minas - MG.

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Vol. 10(28), pp. 2711-2716, 9 July, 2015
DOI: 10.5897/AJAR2014.9332
Article Number: E3CD91253966
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

*Full Length Research Paper*

# Damage in maize ears associated with methods of inoculation of *Stenocarpella maydis*

**Rodrigo Rodrigues Matiello[1]*, Diego Dilson Peretto Miranda dos Santos, Caroline de Jesus Coelho, Maristella Dalla Pria and José Raulindo Gardingo**

Departamento de Fitotecnia e Fitossanidade, Programa de Pós-Graduação em Agronomia da Universidade Estadual de Ponta Grossa, UEPG, 84030-900, Ponta Grossa, PR, Brasil.

Received 13 November, 2014; Accepted 18 June, 2015

**This study aimed to find an efficient method of inoculation of *Stenocarpella maydis* to produce white ear rot (WER) and estimate pathogen damage on maize grain yield components. Measured components were ear mass, grain mass per ear, and thousand grain weight. The experiments were performed in Ponta Grossa, PR, Brazil in a randomized block design with treatments arranged in a split plot with three replications. Three hybrids were studied. For each, five methods of inoculation in the ear at the soft dough stage were compared to un-inoculated controls. The ears were inoculated with 1 mL of the spore suspension ($10^4$ conidia/ml). Evaluations of the disease index (%), severity (%) and lesion area were performed in three (1st experiment) and four periods (2nd experiment), and the area under the disease progression curve was calculated for each of these periods. The area under the disease progression curve was calculated for each period. Inoculation at the center of the ear resulted in the best growth and development of the pathogen in both experiments. Inoculation at the base and center of the ears resulted in greater reductions in yield components, with degrees of damage varying from 27.8 to 43.1%. The inoculation of *S. maydis* in the center of the ear can be considered an appropriate method for resistance screening to WER in maize breeding programs.**

**Key words:** White ear rot, disease progress curve, severity, yield components.

## INTRODUCTION

Fungal diseases (leaf, stalk, root and ear rot) can be observed during all stages of maize (*Zea mays* L.) crop development (Pereira et al., 2005). Maize ear rot diseases are of particular importance because they provide significant reductions in maize production (Duarte et al., 2009). Maize ear rot cause both weight reduction and quality losses in the grain. Some ear rot fungi also produce mycotoxins (Munkvold, 2003) that pose a health hazard to humans and animals consuming cereals products (Mukanga et al., 2010). Ears rots are one of the most dangerous foods and feed safety challenges to maize production in the world (Mesterházy et al., 2012).

The fungus *Stenocarpella maydis* (Berk.) Sutton [syn. Diplodia maydis (Berk.) Sacc.], causal agent of white ear rot (WER), occurs in practically every region of maize cultivation worldwide (Dorrance et al., 1998). *S. maydis* survival between seasons in residue of maize stalks, ears, and fallen grains. Conidia of the fungus are

*Corresponding author. E-mail: rrmatiel@uepg.br, Tel: +554232203752. Fax: + 554232203266.

produced in fruiting structures called pycnidia, which are produced on infested maize residues. When pycnidia is opened the spores are spread by rain splash. In the silking, spores that are splashed up to the ear leaf and then deposited by rainwater around the ear shank have an opportunity to infect (Vincelli, 1997). In Brazil, changes in management practices, like less spacing between rows and monoculture and no-till systems enhance pathogen survival, and led to increases in inoculum quantity (Juliatti et al., 2007). In the Southern region of the country, the favorable environmental conditions promotes to WER development, with medium daily temperatures (25±2°C) and mild nights (12±2°C) resulting in higher incidence of this pathogen on maize crop (Pereira, 1995).

Because maize is the only commercial host of *S. maydis*, crop rotation is considered a viable method for its control (Costa Neto, 1976; Pinto et al., 1997; Reis and Mario, 2003; Reis et al., 2004; Pereira et al., 2005). However, a combination of crop sanitation, good agronomic practices and timely harvesting, has resulted in limited control of WER (Tembo et al., 2013). The utilization of resistant genotypes, therefore, could be a more effective control method, because can promote the field sanity and consequently inoculum reduction (Moremoholo et al., 2010).

Artificial selection strategies for genetic resistance to *S. maydis* could be achieved through breeding programs that aim to produce hybrids with greater resistance to WER. However, to identify resistant genotypes, inoculation methods must be developed to provide the same conditions as natural infection (Kuhnem Júnior et al., 2012). Currently, increasing numbers of maize breeding programs at both public and private institutions are initiating and expanding efforts to develop *S. maydis* resistant hybrids for both human and animal consumption (Mesterházy et al., 2012).

Finding the most appropriate and readily reproducible method of *S. maydis* inoculation will enhance the efficiency of maize breeding programs aimed at the selection of inbred lines and commercial hybrids with the highest level of resistance to this pathogen. The objective of this study was to identify efficient methods of *S. maydis* inoculation in the ears of maize hybrids and to estimate the damage caused by this pathogen by measuring losses in maize grain yield components.

## MATERIALS AND METHODS

### Experimental environments

The experiments were conducted at the "Fazenda Escola Capão da Onça" of the State University of Ponta Grossa, Brazil, during the growing seasons of 2004/2005 and 2005/2006. The soil is classified as Red Yellow Latosol (Brazilian Soil Classification, Embrapa, 2006; and Oxisol, loamy, kaolinitic, thermic Typic Happludox, USDA Soil Taxonomy classification, 2006). The regional climate is classified as Cfb according to the Köppen classification, that is, subtropical humid mesothermal, with cool summers and severe and frequent freezing in the winter and with no defined dry season. The average annual temperature is 17.8°C and annual precipitation is 1,553 mm.

Experimental units consisted of 4 rows, 4.0 m in length with 0.8 m between rows and a seeding rate of 6 seeds per meter. In the experimental areas, no-till was the chosen cultivation system; 300 kg/ha of Nitrogen-Phosphorus-Potassium (NPK) (8-20-20) fertilizer was used, and manual seeding was performed with the help of jab-planters. In the first experiment, seeding occurred on 12/15/2004; seedling emergence on 12/22/2004; and nitrogenized fertilization was performed on the topsoil in a dosage of 200 kg/ha of urea when the plants were at the V2 stage (Fancelli, 1986). The second seeding was conducted on 11/27/2005, and the same cultivation practices used in the first experiment were followed, with seedling emergence on 12/03/2005.

### Experimental design, plant material and inoculation methods

The experimental design was a randomized complete-block in split-plots, with three replications. The hybrids studied in the main plots were provided by the company Dow AgroSciences (8420, 8480 and 2B710), and the effect of ear inoculation: (1) in the rachis, (2) at the base, (3) in the middle, (4) at the tip, (5) sprayed on the style/stigma, and (6) not inoculated (absolute control) were studied in the subplots.

The isolated strains of *S. maydis were given by* Dow AgroSciences. Five discs of a *S. maydis* colony, 5 mm in diameter, were transferred to flasks containing sorghum grains (Silva and Juliatti, 2005) doubly autoclaved at 121°C for 20 min with a 24 h interval between autoclaving according to Mario et al. (2011). The cultures were incubated in a growth chamber at 25 ± 2°C with a photoperiod of 12 h light/12 h dark for 15 days until spores were produced. The inoculum was prepared by adding 200 ml of sterile distilled water to each flask, followed by stirring for the release and formation of a conidial suspension. The spore suspension was filtered through a double layer of cheesecloth, and its concentration was adjusted to $10^4$ conidia $mL^{-1}$. Inoculation was performed with 1 ml of conidial suspension in different parts of the ear with a veterinary-use automatic syringe (50 ml) or sprayed on the style/stigma of the ear with a hand sprayer. The ears were inoculated at the soft dough stage (R4) on 03/24/2005 and 03/15/2006. At inoculations the environment conditions were considered ideals for pathogen development, with medium temperature around 23°C and high relative humidity (> 80%) for both experiments.

### Disease severity and estimate damage

To evaluate the growth/colonization of the pathogen on the hybrid ears, three evaluations were conducted in the 2004/2005 season on 04/05/2005, 04/15/2005, and 04/26/2005, and four evaluations in the 2005/2006 season on 03/29/2006, 04/10/2006, 04/29/2006, and 05/20/2006. For each evaluation, five ears were sampled from the central lines of each sub-plot in the experiments. To assess the severity of the white ear rot, diagrammatic scales and notes were used according to the methodology proposed by Azevedo (1997). Additionally, the length and diameter of the lesions in each ear were measured using a tape measure (cm). The last evaluation of the experiments consisted of the determination of the yield components: ear mass (g), grain mass per ear (g), and thousand grain weight (g). Damage (%) was estimated for these characteristics by comparing the damage encountered in each inoculation method to the damage in the control treatment (without inoculation).

The severity grades were transformed into percentages, representing the Disease Index (DI), through the equation proposed by McKinney (1925), as follows:

$$DI = \sum\left[\frac{(degree\ of\ disease\ severity\ \times frequency)}{(total\ number\ of\ units\ evaluated\ \times highest\ degree\ of\ disease)}\right] \times 100$$

**Statistical analysis**

The area under the disease progress curve (AUDPC) was calculated for the disease index (%), severity data (%), and lesion area of each evaluation according to the equation proposed by Shaner and Finney (1977). The data collected in the experiments were subjected to analysis of variance using the statistical program SISVAR (Ferreira, 2011), and the treatment means, when significantly different, were compared using the Tukey test at 5% probability.

## RESULTS AND DISCUSSION

The *S. maydis* strains obtained from Dow AgroSciences were highly aggressive and allowed the intense growth of white ear rot in the three maize hybrids studied. For both experiments, the AUDPC values for the disease index (DI), severity, and lesion area of WER showed highly significant differences ($p < 0.01$) among the different pathogen inoculation methods. The interaction of the inoculation methods and maize hybrids only demonstrated statistical significance ($p < 0.05$) for the lesion area in both experiments. The lack of a significant effect of the hybrid on the AUDPC characteristics (DI and severity) in the 2004/2005 season may be related to dent grain texture of the hybrids used in this experiment. Mario et al. (2003), evaluating the reaction of six hybrids to WER, found that the hybrids with a dent type grain texture had the lowest incidence of *S. maydis*. The authors also found that the hybrids with dent grain type characteristics produced the highest yields, while the hybrids with a flint grain type had the lowest yields.

Inoculation at the ear rachis and style/stigma spraying were not efficient in the 2004/2005 season, as similar for the non-inoculated control treatment (Table 1). The inoculation of *S. maydis* in the middle of the ear gave the highest disease severity, followed by inoculation at the base of the ear; the disease index was significantly lower when the inoculation was performed at the tip of the ear. Likewise, when the AUDPC was evaluated for severity of the pathogen, inoculation in the middle of the ear proved to be better than the other methods for evaluation of disease (Table 1).

Similar to the results observed in the 2004/2005 season, the AUDPC disease and severity indices were not significantly different for the plants inoculated at the ear rachis and style/stigma spray and the non-inoculated control plants in the 2005/2006 season (Table 1). A significant effect was observed for the interaction of the inoculation methods with the maize hybrids for the AUDPC lesion area in the 2004/2005 and 2005/2006 seasons (Table 2), indicating different behavior of the hybrids in response to different pathogen inoculation methods. For all hybrids, the inoculation of *S. maydis* in the middle and base of the ear led to the largest lesion area, followed by inoculation at the tip of the ear. In both experiments, pathogen inoculation by spraying the conidial suspension on the ear style/stigma appeared to have no effect on any of the evaluated characteristics (Table 2).

Pathogen inoculation by injection of the rachis of the ear did not result in the efficient growth/development of WER in either of the two seasons, with results that were not significantly different from the results of the non-inoculated control. This trend towards decreased development of the pathogen was also observed by Mario et al. (2003) in the method consisting of the deposition of the spore suspension in the leaf sheath behind the ear; in contrast, Bensch et al. (1992) observed a higher incidence of WER through this method of inoculation. Klapproth and Hawk (1991) obtained higher infection levels with the injection of *S. maydis* inside the ear; these results, similarly, showed that the inoculation at the middle and the base of the ear led to the highest levels of pathogen growth. Flett and McLaren (1994) determined the great potential diseases in different maize hybrids inoculating into the apical whorl 2 weeks prior anthesis.

Recently Mario et al. (2011) studied three methods of inoculation with *S. maydis* (natural, spray on stigmas, and deposition on the peduncle) and observed infection rates of 21.0, 39.8, and 44.3% in the ears, respectively. The authors concluded that the inoculation methods consisting of stigma spraying and deposition on the peduncle improved upon the field deposition method of applying the conidial suspension and allowed susceptible hybrids to be differentiated from resistant ones. For the grain yield components (Table 3), highly significant effects of both the hybrids and the inoculation methods ($p < 0.01$) were observed in both seasons for the ear mass (EM), grain mass per ear (GM), and thousand grain weight (TGW). The differences observed in the yield components among the hybrids in both seasons indicate the different yield potentials and genetic backgrounds of the hybrids, with hybrid 2B710 achieving the most positive results.

In both experiments, the inoculation at the middle and base of the ear resulted in the largest reduction in the ear mass, ear grain mass and thousand grain weight (Table 4). The inoculation at the ear rachis and style/stigma spraying resulted in the same yield results as were seen in the control treatment (without inoculation); that is, they did not affect the reduction of the grain yield components (Table 4). These results demonstrate a direct connection between the extent of the disease (disease index, severity and lesion area) and the method of *S. maydis* inoculation. Overall, the inoculation at the middle and base of the ears resulted in the highest pathogen colonization and consequent significant reduction of yield components, whereas the inoculation at the tip of the ear and style/stigma spray resulted in lower colonization of

**Table 1.** Area under the disease progress curve (AUDPC) for the disease index and severity for the different *S. maydis* inoculation methods, 2004/2005 and 2005/2006 seasons. Ponta Grossa-PR, Brazil.

| Inoculation methods | AUDPC - 2004/2005 | | AUDPC - 2005/2006 | |
|---|---|---|---|---|
| | Disease index | Severity | Disease index | Severity |
| Rachis | 0 [d1] | 0 [d] | 0.9 [d] | 1.1 [d] |
| Base | 4.6 [b] | 4.4 [b] | 11.2 [b] | 9.8 [b] |
| Middle | 6.1 [a] | 7.9 [a] | 16.6 [a] | 20.6 [a] |
| Tip | 1.8 [c] | 0.9 [c] | 7.9 [c] | 6.7 [c] |
| Style/stigma spraying | 0 [d] | 0 [d] | 0.3 [d] | 0.3 [d] |
| Control | 0 [d] | 0 [d] | 0 [d] | 0 [d] |
| C.V. (%) | 27.5 | 22.8 | 35.6 | 31.1 |

[1]Means in the same column followed by the same letter do not differ by the Tukey test at 5% probability.

**Table 2.** Area under the disease progress curve (AUDPC) for ear lesion area for the interaction of the inoculation methods and hybrids inoculated with *S. maydis*, 2004/2005 and 2005/2006 seasons. Ponta Grossa-PR, Brazil.

| Inoculation methods | Hybrids - 2004/2005 | | |
|---|---|---|---|
| | 2B710 | 8420 | 8480 |
| Rachis | 0 [A1 c2] | 0 [Ac] | 0 [Ac] |
| Base | 24109.7 [Aa] | 19465.7 [Ba] | 17239.7 [Ba] |
| Middle | 26037.0 [Aa] | 19839.3 [Ba] | 20553.3 [Ba] |
| Tip | 12933.0 [Ab] | 10365.3 [Bb] | 8533.7 [Cb] |
| Style/stigma spraying | 0 [Ac] | 0 [Ac] | 0 [Ac] |
| Control | 0 [Ac] | 0 [Ac] | 0 [Ac] |

| Inoculation methods | Hybrids - 2005/2006 | | |
|---|---|---|---|
| | 2B710 | 8420 | 8480 |
| Rachis | 0 [Cc] | 1910.0 [Bc] | 3468.7 [Ac] |
| Base | 25412.0 [Aa] | 16155.7 [Bab] | 26085.0 [Aab] |
| Middle | 26699.0 [Aa] | 2287957 [Ba] | 29264.7 [Aa] |
| Tip | 11217.7 [Bb] | 11889.0 [Bb] | 19219.3 [Ab] |
| Style/stigma spraying | 1244.0 [Ac] | 0 [Bc] | 1026.0 [Ac] |
| Control | 0 [Ac] | 0 [Ac] | 0 [Ac] |

[1,2]Means followed by the same lowercase letter in the column and uppercase in the row do not differ by the Tukey test at 5% probability.

**Table 3.** Average yield components: ear mass (EM), grain mass per ear (GM) and thousand grain weight (TGW) in relation to hybrids and seasons. Ponta Grossa-PR, Brazil.

| Hybrids | 2004/2005 | | | 2005/2006 | | |
|---|---|---|---|---|---|---|
| | EM (g) | GM (g) | TGW (g) | EM (g) | GM (g) | TGW (g) |
| 8420 | 136.4 [b1] | 110.3 [b] | 224.5 [b] | 143.3 [b] | 121.1 [b] | 238.9 [b] |
| 8480 | 143.7 [b] | 117.2 [b] | 230.5 [b] | 148.3 [b] | 127.2 [b] | 240.7 [b] |
| 2B710 | 165.7 [a] | 137.8 [a] | 270.0 [a] | 166.1 [a] | 140.2 [a] | 265.8 [a] |
| C.V. (%) | 9.4 | 8.8 | 8.1 | 5.7 | 5.2 | 5.8 |

[1]Means followed by the same letter in the column do not differ by the Tukey test at 5% probability.

the ears and thus limited damage to the production components. Silva et al. (2005) reported that *S. maydis* inoculation with a toothpick in the middle of the ear, followed by injection at the base of the ear (R2 stage)

**Table 4.** Average yield components and damage estimates for: ear mass (EM), grain mass per ear (GM) and thousand grain weight (TGW) for different methods of S. *maydis* inoculation, 2004/2005 and 2005/2006 seasons. Ponta Grossa-PR, Brazil.

| Inoculation methods | 2004/2005 | | | | | |
|---|---|---|---|---|---|---|
| | EM (g) | Damage (%) | GM (g) | Damage (%) | TGW (g) | Damage (%) |
| Rachis | 171.3 [a1] | 2.7 | 143.9 [a] | 1.9 | 295.7 [bc] | 13.6 |
| Base | 122.1 [c] | 30.6 | 93.6 [c] | 36.2 | 215.7 [d] | 36.9 |
| Middle | 110.7 [c] | 37.2 | 88.4 [c] | 39.8 | 194.7 [d] | 43.1 |
| Tip | 143.7 [b] | 18.4 | 116.6 [b] | 20.5 | 274.9 [c] | 19.6 |
| Style/stigma spraying | 167.8 [a] | 4.7 | 141.4 [a] | 3.6 | 316.8 [ab] | 7.4 |
| Control | 176.1 [a] | | 146.7 [a] | | 342.2 [a] | |
| C.V. (%) | 7.1 | | 8.7 | | 8.3 | |

| Inoculation methods | 2005/2006 | | | | | |
|---|---|---|---|---|---|---|
| | EM (g) | Damage (%) | GM (g) | Damage (%) | TGW (g) | Damage (%) |
| Rachis | 164.6 [ab] | 8.2 | 138.8 [ab] | 9.9 | 271.9 [a] | 5.8 |
| Base | 127.4 [c] | 28.9 | 106.2 [c] | 31.0 | 208.4 [c] | 27.8 |
| Middle | 126.8 [c] | 29.3 | 104.3 [c] | 32.3 | 206.9 [c] | 28.3 |
| Tip | 145.4 [bc] | 18.9 | 125.5 [bc] | 18.5 | 238.0 [b] | 17.5 |
| Style/stigma spraying | 172.0 [a] | 4.0 | 148.3 [a] | 3.7 | 277.2 [a] | 4.0 |
| Control | 179.3 [a] | | 154.0 [a] | | 288.6 [a] | |
| C.V. (%) | 11.9 | | 11.7 | | 8.0 | |

[1]Means followed by the same letter in the column do not differ by the Tukey test at 5% probability.

provided the greatest AUDPC for this disease; however, inoculation in the middle ear with a toothpick was the only method that resulted in significantly reduced grain yield.

The ear mass was severely affected by the inoculation both at the middle and at the base of the ear. The middle ear inoculation method led to decreases in the ear mass of 37.2 and 29.3% in the first and second seasons, respectively (Table 4). The inoculation at the base of the ear resulted in 30.6 and 28.9% of damage reduction in the first and second experiments, respectively. The rachis and style/stigma spraying inoculation methods resulted in significantly reduced damage estimates, with damage rates between 2.7 and 8.2% for the inoculation in the ear rachis. These estimates may reflect the low amount of disease (DI, severity, and area of injury) associated with *S. maydis* inoculation in the rachis and style/stigma spraying (Table 4). Mario et al. (2003) reported that spraying the conidial suspension on the style/stigma of the ear caused significant reductions in the average grain yield of the inoculated hybrids; these results differ from the results obtained in our study for style/stigma spraying.

The results of both experiments confirmed the negative effect of the pathogen on the ear grain mass, particularly when the inoculation occurred at the middle and at the base of the ear. For the first experiment, while an average grain weight of 146.7 g per ear was achieved in the control treatment, the inoculation at the middle of the ear resulted in a mass of only 88.4 g (-39.8%). Likewise, the damage reduction in the second season was estimated at 32.3% for the grain weight (Table 4). Additionally, a significant reduction in TGW was observed for the plants inoculated at the middle or at the base of the ear, with the damage estimates ranging from 27.8 (base) to 43.1% (middle) (Table 4).

The hybrids used in our experiments (8420, 8480 and 2B710) have grain textures ranging from semi-dent to flint. In a study by Mario et al. (2003), the hybrids obtaining the highest yields and the lowest incidences of infection with *S. maydis* were dent grain characteristics, while the hybrids with a flint grain were the most affected by the pathogen (greater incidence and severity), with a significant reduction in the grain yield.

The results obtained in this study emphasize the vulnerability of maize ears to *S. maydis* infection at the soft dough stage (R4). In fact, the soft dough stage is the ideal period for differentiating susceptible from resistant germplasms (Chambers, 1988) and also an auxiliary tool in determining the disease potentials (Flett and Mclaren, 1994). Thus, significant reductions in the measures of yield are associated with inoculation methods that allow further growth of the lesion, including inoculation at the middle and the base of the ear. It is important to identify the most effective method of inoculating *S. maydis* so that high levels of resistance to the pathogen can be selected in inbred lines and hybrids. The results obtained in this study indicate that inoculation at the middle and the base of the ear are the most promising methods for identifying variants with resistance/susceptibility to this pathogen.

## Conflict of Interest

The authors have not declared any conflict of interest.

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Vol. 10(28), pp. 2717-2723, 9 July, 2015
DOI: 10.5897/AJAR2015.9737
Article Number: 0EE4ABF53968
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

*Full Length Research Paper*

# Perception of buyers in regards to the quality and food safety of minimally processed vegetables

**Michelle da Rosa Lopes, Francisco de Assis Rolim Pereira, Celso Correia de Souza, José Francisco dos Reis Neto* and José Antonio Maior Bono**

Department of Master in Production and Agro-industrial Management, University Anhanguera Uniderp. Rua Alexandre Herculano, 1400, CEP 79037-280, Campo Grande, MS, Brazil.

Received 19 March, 2015; Accepted 18 June, 2015

**This article is based on the buyer's general behavior with the objective of identifying the consumption characteristics of minimally processed vegetables. A descriptive approach was used to understand how buyers make their decisions on the day-to-day, with a non-probabilistic sample of 328 questionnaires employed at the largest circulation areas of the seven administrative regions of Campo Grande (MS), Brazil. The data was analyzed using parametric and nonparametric statistical techniques in order to understand the perceptions and demands of buyers during the decision-making process of purchasing vegetables. The key results show strong evidence of the consumption of vegetables, especially from elder buyers (66.7% every day). The vegetables are selected using the perception of appearance and price, while buyers indicate that the product brand is less relevant. It stood out that the higher the consumption, the greater consumer awareness about the quality, certification and food safety requirements of vegetables. It was demonstrated that buyers demand a greater product quality and food safety, and are willing to pay more for it; however, they report that they do not read labels or product information. The managerial implications present empirical information relevant to vegetable sellers and the inclusion of marketing strategies.**

**Key words:** Vegetable consumption, certification of vegetables, consumer behavior, origin of vegetables.

## INTRODUCTION

A food product when launched in the market, even if involuntarily, undergoes consumer analysis before being acquired. The criteria used vary, but generally the healthy characteristics of the product are considered, which in addition to its appearance are reinforced by information labels, origin and processing. Thus, the consumer behavior during the purchase is complex and interactive. The purchase of a product is influenced by the perception of its use and its value (Ferreira et al., 2010).

The production chain of minimally processed vegetables is characterized by the strong influence from the final consumer, once the details of the products' appearances constitute significant facts for the purchase to be completed. As with other segments of agribusiness, the desire and requests of buyers must always be considered. According to data from a survey by Silveira et al. (2011), the annual vegetable consumption per person in Brazil in 2002 was 29 kg, decreasing to 27 kg

*Corresponding author. E-mail: jfreisneto@uniderp.edu.br

in 2008. The Midwest and the South (unlike the Southeast) were the regions where consumption of vegetables increased, reaching 28.6 kg per capita in 2008.

In Campo Grande (MS), according to data from Núcleo de Estudos e Pesquisas Econômicas e Sociais {Center of Economic and Social Studies and Research} (NEPES, 2013), the family budget has a share of 24.86% allocated to food, with vegetables contributing to 2.76%; in other words, 11.10% of the cost of food for a typical family in Campo Grande (MS), Brazil.

Analyzing consumer behavior and involvement in the buying process is extremely important for the sectors of production, processing and sale. According to Giglio (2013), there are means to identify customer demands and which strategies should be adopted in the consumer market. According to Amaral et al. (2007), the appearance, image and how the company communicates with consumers, determines its position in the market. Schiffman and Kanuk (2009) state that consumers make buying decisions taking into account their available resources, such as time, money and physical effort. It is a perceptive process for the consumer, who receives stimuli combined with motivation, emotion and expectation, interprets and processes the information received, and is encouraged and driven to carry out the economic transaction, or not.

The vegetables studied in this paper are the minimally processed, packaged, sanitized and available vegetables in a point of sale for consumer purchase. For Perez et al. (2008), minimally processed vegetables emerged as an interesting alternative for the consumer that does not have much free time. They are modified physically, maintaining the state of freshness, and have the appearance of a product with quality and safety.

Some aspects of the purchase of vegetables are classified by Lourenzani and Silva (2004): freshness (characteristic of quality accumulated from production, harvesting, handling, storage, exposure to consumption; considering the appearance, color, hygiene, etc.), image, price, convenience, types of packaging, availability of information, and food safety. With this same focus, Neves et al. (2011) point out (in order of importance) what consumers of vegetables consider in the purchase decision-making process: freshness, nutrition, taste, safety, price, and convenience. In regards to the aspects of the product, they report that consumers consider that food safety precedes the nutritional value, and follows: the potential lack of food, taste and appearance. In summary, the authors portray the additional concern of consumers regarding the safety and quality of food products available to them.

It is understood by Lopes et al. (2011) that the segregation of food into distribution groups is important, as customers have different needs and present different trends. Blackwell et al. (2005) complemented that consumers go through the process of recognizing a need, searching for information, evaluating alternatives, pre-purchase, purchase, consumption, post-purchase evaluation, and disposal. Marketing professionals need to be aware of this process in order to understand their consumer.

According to Silva and Machado (2009), the distribution channels of vegetables must be related to the consumer's daily life, after studying their preferences and influencers. Vegetables consumers, especially those who buy in supermarkets, consider the following factors: importance of food, product diversification and annual variety, exhibition, portion sizes, processing, cleanliness, and origin (Alcântara and Souza, 2009). These considerations are strengthened by Gains' (1994) proposed model cited by Batalha et al. (2009), in which food consumption behavior occurs with the interaction of three factors: food, consumer, and the context of consumption. These are related to food, influences of the smell, taste, flavor, nutrients and packaging. The following variables are associated with the consumer: habits, culture, personality, humor and psychology. Some attributes such as the conditions under which the vegetables were produced, are not perceived by consumers. Therefore, ensuring respect and responsibility for food will be a great challenge for entrepreneurs and producers, having to standardize processes to create a sense of confidence for the consumer Alcântara and Souza (2009).

In the scheme suggested by Spers (2011), the consumer perceives some attributes related to food and classifies them into extrinsic qualities (easily perceived by the consumer by the characteristics of the external environment, such as: quality label, brand, appearance, etc.) and intrinsic (product quality, food safety, nutrition, etc.). When making the purchase decision, the aspects that were assessed only by factors such as price, convenience and appearance, are now assessed by extrinsic and intrinsic qualities (Spers, 2011).

For Vitti et al. (2011), the evaluation of consumer perceptual values becomes important for the effective completion of an economic transaction. The vegetable supplier is responsible for evaluating what is being presented as a product, its image and promotion. Vegetables are very important for the human health, contributing to various vitamins and essential salts for the proper functioning of the body, and helping to prevent various diseases (Coelho, 2007). Therefore, one cannot ignore the importance of consuming vegetables on a daily basis. Consumers should be aware of its quality and safety.

In regards to the aspect of quality, the consumer evaluates the primary elements, such as those proposed by Garvin (1992): perceived quality (comparative advantage of similar products or substitutes), compliance (initial promised characteristics), reliability (consumer experience and proof of promised characteristics under compliance), aesthetic (smell, taste, appearance), durability (product life) and service (quick and easy to acquire the vegetable). The aspects of perceived quality, compliance, reliability, and aesthetics, may be linked to the perception of vegetable consumers in regards to the use of chemicals in the production, conservation and visual.

According to Ventura (2010), there are significant changes in the consumer profile. New markets are growing and others must be rescued. The growing effort to improve quality of life can be perceived by the increased consumption of healthy products and services, the most obvious ones being food products. This study aimed to identify the perception and demands of consumers in Campo Grande (MS), Brazil, in the purchase of minimally processed vegetables, and to identify the attributes that influence during the decision-making process of acquiring such foods.

## MATERIALS AND METHODS

The study was conducted in the city of Campo Grande (MS), Brazil, during the period of 1st May to 30th 2013. In order to meet the research objectives, a descriptive approach was used to understand how consumers make their purchasing decisions in the day-to-day, using a non-probabilistic sample constituted by the economically active population (EAP). According to data from IBGE (2013), out of the 832,352 people estimated for 2013, it was estimated that 454,404 was part of the EAP. The sample size was defined according to Fonseca and Martins (2006), considering the nominal variable and the finite population, with a confidence level of 95% and a sampling error of 7%, with the positive response rate of 0.50 of one of the research variables, making up a total sample of 328 individuals.

The scale adopted for the perceived importance of the interviewee was the seven-point Likert scale, ranging from 1. Not Important; 2. Irrelevant; 3. Not very relevant; 4. Neutral (undefined); 5. Not very important; 6. Important; 7. Very important. The other scales adopted were nominal and ordinal for the establishment of demography and vegetable consumption practices. The scales were developed by Amaral et al. (2007) and Batalha et al. (2009), with the adjustments made by the authors. The questionnaire and the project were sent to evaluation and approval of the Ethics Committee (Approved on 04/09/2013, protocol No. 246.304).

To analyze the consumer behavior in relation to the perception of food quality and safety of minimally processed vegetables, field research was carried out with a questionnaire divided into three parts: In the first part, a survey was conducted in relation to the consumer profile (gender, age, education level, and family income); the second part consisted of the perceived quality of vegetables; and the third part was comprised of questions about consumer behavior when acquiring vegetables. The questionnaire was applied to consumers in the major areas of people circulation of the seven administrative regions of Campo Grande city. The research had a social aspect, with voluntary and confidential interviews. The data was tabulated and processed in the Sphinx Lexical 5.0 and SPSS 21.0 softwares, applying univariate and bivariate analysis, obtaining stratification of consumers in relation to the usual signs of use.

## RESULTS AND DISCUSSION

A data consistency analysis was conducted in order to find atypical or missing data, followed by parametric and non-parametric statistics analysis, when tolerable by the adopted scales.

### Consumer profile for minimally processed vegetables

The results showed that the consumer profile for minimally processed vegetables in Campo Grande is made up of 57.9% of females; the most representative age group was between 18 and 34 years old, totaling 62.2% of respondents, and only 10.9% have a high school degree and 37.8% have a university degree. It is observed that 40.9% of respondents had an income of up to R$1,244, and 51.9% had an income between R$ 1,244 and R$6,220 (Table 1). The profile of respondents coincide with the characteristics of the new middle class, which has been growing in the country as a whole, changing the buying behavior and intensifying the perception of product quality, safety, and available information (SAE, 2013).

### Frequency of consumption and consumer perception regarding the certification of minimally processed vegetables

It was sought to identify the main characteristics of perceptions relevant to the acquisition of vegetables. In order to evaluate the degree of dependence between the segmentation of vegetables consumers and the frequency of consumption (Castro and Neves, 2011), the chi-square test for non-parametric variables was used. The results are shown in Table 1.

It was found that for the chi-square test of independence ($p<0.05$), there were no significant associations between the frequency of consumption and the following segmentation variables: gender, education level, and family income. Segmentation by age group showed significant dependence on the frequency of consumption ($\chi^2 = 21.096$, $p = 0.012$). It is supposed that consumers within the ages of 35 to 60 years consumed more vegetables than younger consumers up to 34 years old. This conclusion contradicts Ventura (2010), which he reports that younger people purchase more vegetables than older people. As shown in Table 2, it was observed that those buyers who knew the meaning of food certification and those who buy certified products, are associated with the frequency of consumption of vegetables. The research showed that the higher the frequency of vegetable consumption by the consumer, the greater the indication of the perception of certification (52.9%) and the greater the purchase of certified products (48.3%). These results can contradict those found by Perez et al. (2008), who claim there is no significant association between consumers who know what certification is and consumers who buy certified

**Table 1.** Consumer profile of buyers and the frequency in consumption of vegetables.

| Variables | | n | Frequency in consumption of vegetables (%) | | | | Pearson chi-square test | |
|---|---|---|---|---|---|---|---|---|
| | | | Every day | 3 times a week | 2 times a week | Once every 15 days | $\chi^2$ | p |
| Gender | Male | 138 | 41.3 | 30.4 | 18.1 | 10.1 | 3.203 | 0.361 |
| | Female | 190 | 51.1 | 25.3 | 14.2 | 9.5 | | |
| Age (years) | ≤24 | 109 | 36.7 | 30.3 | 21.1 | 11.9 | 21.096 | 0.012 |
| | 25 to 34 | 100 | 39.0 | 31.0 | 18.0 | 12.0 | | |
| | 35 to 49 | 83 | 61.4 | 22.9 | 9.6 | 6.0 | | |
| | ≥50 | 36 | 66.7 | 19.4 | 8.3 | 5.6 | | |
| Education level | Primary | 57 | 47.4 | 29.8 | 14.0 | 8.8 | 9.741 | 0.372 |
| | Secondary | 134 | 44.0 | 28.4 | 20.1 | 7.5 | | |
| | Bachelor | 124 | 46.8 | 27.4 | 12.9 | 12.9 | | |
| | Pos-Graduate | 13 | 76.9 | 7.7 | 7.7 | 7.7 | | |
| Family income (R$) | ≤ 1,244 | 134 | 40.3 | 29.1 | 18.7 | 11.9 | 7.842 | 0.550 |
| | 1,244 to 2,487 | 96 | 47.9 | 28.1 | 16.7 | 7.3 | | |
| | 2,488 to 6,219 | 74 | 52.7 | 24.3 | 13.5 | 9.5 | | |
| | ≥$6,220 | 16 | 62.5 | 25.0 | 4.2 | 8.3 | | |

Legend: n – number of respondents in the segment; R$1 =US$0.31.

**Table 2.** Frequency in consumption of minimally processed vegetables vs. knowledge of what certified food product is and if they would buy products with a certification of origin.

| Variables | | Frequency in consumption of vegetables (%) | | | | Pearson chi-square test | |
|---|---|---|---|---|---|---|---|
| | | Every day | Times a week | 2 times a week | Once every 15 days | $\chi^2$ | p |
| Knows what certified food product is | Yes | 52.9 | 27.2 | 12.6 | 7.3 | 9.309 | 0.025 |
| | No | 38.7 | 27.7 | 20.4 | 13.1 | | |
| Would buy products with a certification of origin | Yes | 48.3 | 28.6 | 15.0 | 8.2 | 11.209 | 0.011 |

products.

Table 3 shows that the higher the frequency of consumption of vegetables by the consumer, the greater the awareness of the importance of quality in vegetables. The Student *t* test was used for the extracted data from the Likert scale of 7 points. Consumers who indicated they consumed vegetables every day also affirmed that this attitude is very important, since an average of 6.8 was obtained from the questions on the Likert scale 1-7 (1 = not important to 7 = very important). The $\chi^2$ test indicated that there was a highly significant association between consuming vegetables every day and its importance for health ($p < 0.001$). This statement portrays that consumers consider the quality of vegetables in their daily consumption to be important.

## Information on label and quality of minimally processed vegetables

Buyers deemed that the information on the packaging labels of vegetables was important, such as: production, transportation, and processing; as indicated in the seven-point scale of importance (1 = not important to 7 = very important), obtaining an average of 6.1 points and a standard deviation of 1.37. These values portrayed the importance of labels in vegetables.

In regards to the understanding of food safety of vegetables, 84.5% of buyers demonstrated knowledge of the application of pesticides in vegetable production; 91.2% believe that their intensive or indiscriminate use can harm the health of human beings; and 70.4%

**Table 3.** Frequency in consumption of minimally processed vegetables vs. the importance of the quality in vegetables for its consumption.

| Variables | | Frequency in consumption of vegetables | | | | Student *t* test | |
|---|---|---|---|---|---|---|---|
| | | Every day | 3 times a week | 2 times a week | Once every 15 days | *t* | p |
| Importance of the quality in vegetables for its consumption | Average | 6.8 | 6.3 | 5.7 | 5.3 | 24.738 | 0.001 |

reported they can choose between vegetables produced by the conventional system versus the organic system due to information in points of sale. 78.4% of buyers would like to get information about the production cycle, transporting, and processing of vegetables. These values are related to the proposals presented by Castro and Neves (2011) and Alcântara and Souza (2009), which report consumer interest in vegetables related to safety information on the label and vegetables quality.

## Types of minimally processed vegetables and where to buy

In regards to preferences for vegetables, this matter was addressed with 15 alternatives represented by 15 common vegetables with multiple choices of up to five options. The main vegetables bought/preferred by consumers were: lettuce (*Lactuca sativa*) with 17.6%, tomato (*Solanum lycopersicum*) with 13.4%, carrot (*Daucus carota*) with 11.4%, beet (*Beta vulgaris esculenta*) with 8.7%, and chives (*Allium schoenoprasum*) with 8.2%. Consumers reported purchasing vegetables in the city's traditional points of sale. In a multiple-choice question (up to three options), 33.1% of consumers preferred supermarkets, 26.6% preferred street markets, and 14.7% chose grocery stores/among others.

## Consumer perception and main considerable attributes during the acquisition of minimally processed vegetables

Eight attributes were analyzed in regards to its importance in the vegetable purchasing decision: appearance, brand, organic vegetables, conventional vegetables, origin, place of purchase, packaging, and price. The results are presented in Table 4. When the attributes of vegetables in function of the frequency of consumption were evaluated, it appears that there are significant differences in the appearance, or freshness, among vegetables. The results indicate that these attributes are more important for those who indicated a higher frequency of consumption. Respondents who consume vegetables every day indicated on the Likert seven-point scale that appearance was the most important attribute (mean = 6.62). When the results were segmented by buyers' gender, instead of frequency of consumption, only the attribute of price showed averages with significant differences at 5% ($t = 2.404$ and $p = 0.017$). Women give more importance to price (mean = 6.17 and standard deviation = 1.383), than men (mean = 5.78, standard deviation = 1.608).

## Willingness to pay more for a minimally processed vegetable containing food quality

It was proven that 66.7% of consumers are willing to pay more for a vegetable according to its label information about food quality and safety, even though the dependence was insignificant ($\chi 2 = 3.673$, $p = 0.597$). About 53% of consumers said they were willing to pay 5% to 10% more than the traditional value for a vegetable with proof of origin, as this proved to be an important factor. It is concluded that consumers perceive the value, not the price, provided that sufficient information is obtained in regards to the choice of vegetables with food safety and product quality. This finding is substantiated by what Castro and Neves (2011) presents, in that the certified vegetable is a value perceived by the consumer.

In the diet of the average Campo Grande resident, the consumption of vegetables is substantial mainly for seniors. It was observed that there is a consumer concern in regards to food safety and product quality, even though half of these consumers reported that they do not read labels. The supplying of vegetables with information about origin and handling is important and influences the consumer's willingness to pay more. The attributes of appearance, organic vegetables, and conventional vegetables are factors that attract purchases. When gender was analyzed, only the price of vegetables was shown as significant. The results of this study reveal some relevant considerations for business owners in regards to the sale of vegetables in supermarkets, grocery stores and street markets; the results showed that the consumer is willing to pay more for vegetables if there is reliable information regarding its production, handling, transportation, and availability. In

**Table 4.** Descriptive statistics of the attributes of vegetables and multiple comparisons in relation to the variable.

| AVVVVVV Variables VVVARIBLE | Frequency in consumption of vegetables | | | | | | | | Significant differences at p<0.05 between the averages of groups of frequency in consumption |
|---|---|---|---|---|---|---|---|---|---|
| | Daily | | 3 times a week | | 2 times a week | | Once every 15 days | | |
| | Group A, n=154 | | Group B, n=52 | | Group C, n=90 | | Group D, n=24 | | |
| | Average | Standard deviation | Average | Standard deviation | Average | Standard deviation | Average | Standard deviation | |
| Appearance | 6.62 | 0.834 | 5.90 | 1.432 | 6.18 | 1.312 | 6.13 | 1.296 | A & B, A & C |
| Brand | 4.58 | 1.950 | 4.29 | 1.764 | 4.21 | 1.777 | 3.50 | 1.911 | nsd |
| Organic vegetables | 6.01 | 1.391 | 5.08 | 1.770 | 5.49 | 1.831 | 5.13 | 2.071 | A & B |
| Conventional Vegetables | 5.49 | 1.581 | 4.92 | 1.835 | 5.10 | 1.656 | 4.29 | 1.574 | A & D |
| Origin | 5.95 | 1.450 | 5.42 | 1.601 | 5.61 | 1.548 | 5.38 | 2.081 | nsd |
| Area of commercialization | 5.73 | 1.626 | 5.69 | 1.449 | 5.69 | 1.548 | 5.46 | 1.444 | nsd |
| Packaging | 5.72 | 1.623 | 5.56 | 1.602 | 5.44 | 1.690 | 5.58 | 1.442 | nsd |
| Price | 6.15 | 1.404 | 6.12 | 1.278 | 5.92 | 1.501 | 5.96 | 1.367 | nsd |

Legend: nsd – no significant difference at p<0.05, between the groups of frequency in vegetable consumption.

addition, the consumer recognizes the existence of food safety and product quality. The entrepreneur's effort in the use of marketing capabilities has prospects of an assured return if supported by the perceptions of the main attributes considered in the choice of vegetables.

## Conclusion

The consumer of vegetables values products containing information about its production and post harvest processing. Product quality combined with the perception of food safety convinces the consumers to pay more for vegetables.

## Conflict of Interest

The authors declare they have not conflict of interest.

## ACKNOWLEDGMENTS

Thanks to the University Anhanguera Uniderp for assisting with infrastructure and personnel and to the Fundação Manoel de Barros (FMB) for the financial support to perform this research.

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academicJournals

Vol. 10(28), pp. 2724-2730, 9 July, 2015
DOI: 10.5897/AJAR2014.9356
Article Number: 6AAAE9F53971
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

*Full Length Research Paper*

# Comparison between manual and semi-mechanical harvest of coffee fruit in mountainous areas

**Gilberto Rosa de Sousa Filho[1], Henrique Duarte Vieira[2]*, Weverton Pereira Rodrigues[2] and Fábio Luiz Partelli[1]**

[1]Universidade Federal do Espírito Santo (UFES), Centro Universitário Norte do Espírito Santo, Rod. BR 101 km 60, 29932-540 Bairro Litorâneo, São Mateus, Espírito Santo, Brazil.
[2]Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Centro de Ciências e Tecnologias Agropecuárias, Avenida Alberto Lamego 2000, 28013-602 Parque Califrórnia, Campos dos Goytacazes, Rio de Janeiro, Brazil.

Received 19 November, 2014; Accepted 18 June, 2015

**In the coffee production, the most expensive operation is the harvest, mainly by lack the labor this time of year. In regions of large and flat areas, the use of mechanization allows a good reduction in the production cost. In mountainous areas the coffee harvest is performed manually. With the aim of reducing production costs, there has been a continuous increase in the use of machinery, especially at harvest. In mountainous regions the semi-mechanized harvest is already being used by aid of portable harvesters that harvest the coffee quickly and at low cost. Family agriculture dominates in Brazil and the use of portable harvesters in small farms can contribute to income increase. This study evaluated manual and semi-mechanical harvest of coffee fruit in a mountainous region of northwest Rio de Janeiro State. The experiment was conducted in June 2012 on arabica coffee crop, cv. Catucaí Vermelho 785/15, spaced 0.5 × 2.2 m, three years old, without irrigation, with a height and yield average of 1.75 m and 72 bags ha$^{-1}$, respectively. Results showed that the use of portable harvesters yielded up to ca. eight times more than manual harvest. This is probably due to the high productivity presented by this crop, fruit ripening uniformity and the small amount of green fruit. The semi-mechanized harvest showed a cost reduction of 27% when compared to manual, it also showed the highest efficiency, but had the highest leaf drop, more than 33%. Other studies should be performed to identify the level at which leaf drop is detrimental to coffee productivity, in this condition.**

**Key words:** *Coffea arabica* L., performance, operational cost.

## INTRODUCTION

In recent years, coffee production has exceeded 8 million tons of coffee, mainly from the countries of South America and Asia (ICO, 2014a). It is estimated that coffee production chain, from the export and processing to the final product for consumption, generates a global income of *ca.* US$ 173.4 billion (ICO, 2014b), constituting

*Corresponding author. E-mail: henrique@uenf.br, Tel: +552227397049.

the social and economic base of many countries.

Among the management practices related to coffee production, the harvest is an operation that requires large costs in production, accounting, on average, 30% of the total cost and about 50% of the labor employed (Matiello et al., 2009). Regardless of the harvest system used, the detachment of fruits is the most complex operation, representing 75% of the time spent when using the manual system (Bártholo and Guimarães, 1997). Therefore, it becomes important to use practices which maximize harvest operation such as mechanized and semi-mechanized harvest.

The use of coffee mechanical harvest increases the operation efficiency, contributing to cost reduction (Cassia et al., 2013) and, furthermore, decreased harvest time reduces fruit exposure to the environment, which contributes to increased drink quality, if care is taken in the following steps (Silva et al., 2006). However, the slope of the coffee cultivated in coffee mountainous regions, that are present in many of the countries of South America, Asia and Africa, hinders mechanization.

Aiming to improve harvesting efficiency, small and medium producers have used a semi-mechanized system termed portable mechanical coffee harvesters, which have yields up to eight times higher than manual harvest (Silva et al., 1997), and also exhibits itself as a viable solution to the mountainous regions. Besides supplying the labor shortage, the adoption of this type of machine can improve workers' pay, since its use and maintenance requires higher technical qualification (Souza et al., 2006).

Portable mechanical coffee harvesters have been driven by pneumatic system, internal combustion or electric motor. The drive potencies of these systems are above 735 W for pneumatic systems and internal combustion motors and 500 W for electric motors (Barbosa et al., 2005).

The use cost of an agricultural machine is divided into fixed and variable costs. Fixed costs are those that do not vary with the use intensity of the machine, citing depreciation, interest on invested capital, taxes, cost associated with machine insurance and shelter, while the variable costs are influenced by intensity use of machine and involve costs associated with fuel and lubricants, repair and maintenance costs and the labor to operate the machine (Balastreire, 1987).

Despite presenting as a great option for small and medium producers and for mountainous areas, portable mechanical coffee harvesters need to be widely studied, because there are few studies related to the matter, such as those performed by Barbosa et al. (2005). Thus, it is possible to improve the information on how conditions related to the crop (spacing, height, yield, maturity, and other factors) may influence the efficiency and cost of semi-mechanized harvest. Thus, the objective of this study was to compare the manual and semi-mechanized harvest methods with emphasis on performance and operation cost.

## MATERIALS AND METHODS

The experiment was conducted in June 2012, in a mountainous region in the northwest Rio de Janeiro state, Brazil, with an average annual temperature of 19°C, with winter minimum temperatures averaged at 14.2°C, and summer maximum temperatures averaged at 24.6°C. Two treatments (manual and semi-mechanized harvest) were applied on, three years old, Catucaí Vermelho 785/15 (*Coffea arabica* L.) cultivar plants, spaced 2.2 × 0.5 m, managed without irrigation, with a height and yield average of 1.75 m and 72 bags-60 kg $ha^{-1}$, respectively and 96.6% of ripe fruit.

Manual harvest was performed by four workers, which separated the fruits from dropped leaves after fruit detachment. In turn, the semi-mechanized harvest was performed by three workers, one operated the portable mechanical coffee harvester (M230 Shindaiwa 22.5 displacement model, 1.2 hp, 4.2 kg, – Figure 1), while the other two workers did the manual repass (manual removal of the fruits that don't detached by the action of portable machine). All contributed to the separation of leaves and fruits. In both methods, the coffee fruits were harvested on cloth. After harvest, the volume of harvested coffee (in liters) and weight of dropped leaves were measured, with the aid of a portable digital scale.

The statistical design used, was complete randomized blocks with two treatments (manual and semi-mechanized harvest) and six blocks (three morning and three in the afternoon), each plot containing 20 plants. The blocks were separated by four planting rows, and the first 20 plants each row were used as border. In each plot harvest time (HT), time of leaf separation (TS), volume of harvested coffee (HC), weight of dropped leaves (WFL), were measured.

From these data it was possible to calculate: the volume of coffee cherries harvested by worker (VH), the volume of harvested coffee cherries per worker per hour (HHV) and the amount of harvested coffee per worker per hour, considering the time of leaves separation (VHHn).

To determine the operational cost of portable mechanical coffee harvesters we used the following methodology: linear depreciation for a lifespan of 2,500 h, interest rate of 4% pa, cost associated with machine insurance and shelter of 2%, fuel and lubricating oil according to field measurements, repair and maintenance according to maintenance plan from the manufacturer's manual and worker's salary (for both manual and semi-mechanized methods) of US$ 17.93 (including social charges and value at the time of experiment).

The variables were subjected to analysis of variance and means were compared by Tukey test at 5% probability. All analyzes were performed using the statistical analysis program Genes (Cruz et al., 2013).

## RESULTS AND DISCUSSION

There were significant differences between harvest methods for HT, VH, VHH, VHHn, WFL and cost of harvest variables. For TS and HC no significant differences were observed (Table 1). The manual harvest showed a higher HT than semi-mechanized (approximately 50%), indicating a potential for reducing the collection time in the semi-mechanized method

**Figure 1.** Portable mechanical coffee harvester used in the experiment.

**Table 1.** Summary of analysis of variance and variation coefficients (CV) for harvest time (HT); time to separate leaf (TS), volume of harvested coffee (HC), gross volume of harvested coffee per worker (VH), gross volume of harvested coffee per worker per hour (VHH), net volume of harvested coffee per worker per hour (VHHn), weight of fallen leaves (WFL) and operational cost.

| SF | HT[1] | TS[1] | HC[1] | VH[1] | VHH[1] | VHHn[1] | WFL[2] | Cost[3] |
|---|---|---|---|---|---|---|---|---|
| Bl | 2.93 | 0.93 | 28.75 | 3.68 | 511.62 | 205.80 | 414.92 | 32739.08 |
| Trat | 56.36** | 0.33$^{NS}$ | 2.08$^{NS}$ | 133.80* | 24301.80** | 6474.20** | 40194.19** | 213600.87** |
| Res | 1.33 | 4.67 | 160.48 | 14.65 | 235.45 | 92.48 | 553.19 | 9947.09 |
| CV % | 10.66 | 15.66 | 16.39 | 16.97 | 11.38 | 11.54 | 5.81 | 8.83 |
| Average | 10.83 | 6.17 | 77.25 | 22.54 | 134.78 | 83.34 | 404.46 | 1129.39 |

d.f. = degrees of freedom; *, **significant at 1 and 5% probability, respectively; [1] in minute; [2] in Kg; [3] In US\$ ha$^{-1}$.

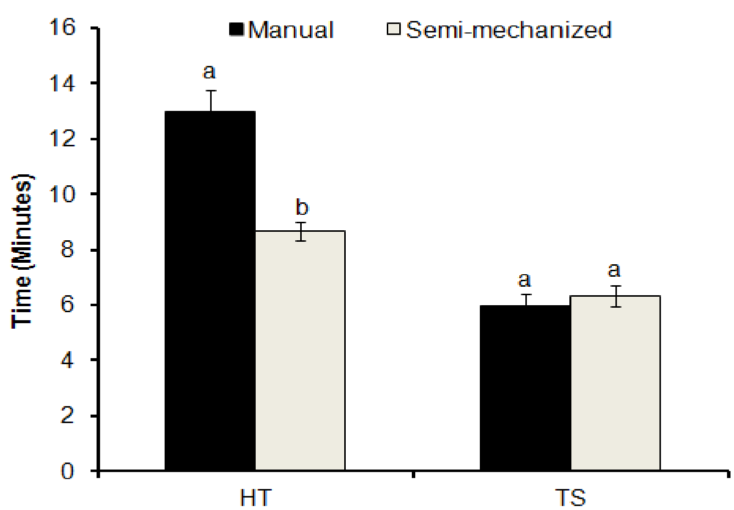


**Figure 2.** Harvest time (HT) and time to separate leaf (TSL) in manual and semi-mechanical harvest of coffee fruit. Means followed by the same letter do not differ by the Tukey test at 5% probability. Each value represents the mean ± S.E. (n=6).

(Figure 2). As both harvest methods showed the same TS, the HT was what significantly influenced the other variables.

The HT is highly influenced by crop conditions, especially productivity, plant height and green fruits percentage and also by machine setting conditions

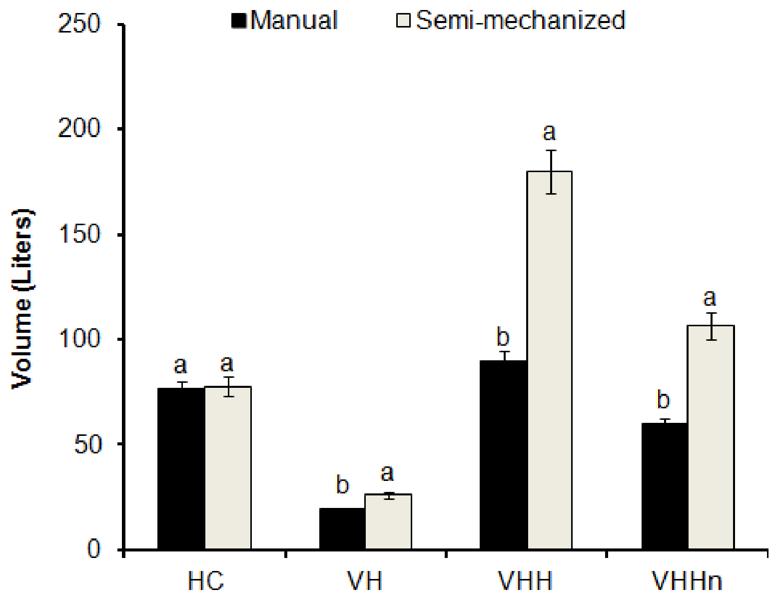


**Figure 3.** Harvested coffee volume (HC), gross volume of harvested coffee per worker (VH), gross volume of harvested coffee per worker per hour (VHH) and net volume of harvested coffee per worker per hour (VHHn) in manual and semi-mechanical harvest of coffee fruit. Means followed by the same letter do not differ by the Tukey test at 5% probability. Each value represents the mean ± S.E. (n=6).

(Barbosa et al., 2005; Souza et al., 2006). A large percentage of green fruits can reduce the volume of detached fruits, by machine, forcing the operator to spend more time in each plant, in order to detach the greatest possible amount of fruits, which ultimately contributes to reducing the harvest efficiency, through portable mechanical coffee harvester. In this work, the green fruit percentage of the plant was only 3.4%, indicating that the crop showed a good homogeneity of mature fruits, when it was decided to start the harvest, favoring the semi-mechanized harvest, this is also observed in mechanized harvest (Silva et al., 2006). Furthermore, reduction of HT increases the possibility of improving the coffee drink quality (Silva et al., 2006).

Although coffee quality analyzes were not made, there is the possibly that it would be the same between treatments, as observed by Carvalho Júnior et al. (2003) which did not find differences in the coffee quality in the various harvest methods, including what we carried out in this study, manual compared to semi-mechanized harvest with aid of portable mechanical coffee harvester.

Both harvest methods showed similar values of HC (Figure 3), indicating a good homogeneity of the crop and thus differences in other variables were closely related to harvest methods. Considering the number of workers involved in each method, the VH to semi-mechanized was approximately 35% higher than the manual method (Figure 2), indicating the highest yield for the first method. It is more evident when considering the VHH, wherein the semi-mechanized method was superior in more than 100% (Figure 3). However, when considering the VHHn, semi-mechanized was approximately 78% higher than manual method (Figure 3). Barbosa et al. (2005) evaluated different settings to the portable mechanical coffee harvester and they found highest yield from 100 until 150% for semi-mechanized compared to manual method for a crop with plants of 1.3 m height and 6.27% green fruit. These differences may be related to the plants conditions, work speed of workers, machine setting conditions and machine operator. According to Silva et al. (1997), the performance of portable mechanical coffee harvester can be up to eight times higher than manual method.

The use of machines for coffee harvest beans can cause damage to the plant, such as defoliation, branches (plagiotropic branches) and trunk apex breaking (orthotropic branch) (Aristizábal-Torres et al., 2003). In this work, the WFL to semi-mechanized was 33% higher than manual method (Figure 4).

According to Bártholo and Guimarães (1997), the defoliation caused in the coffee, by the use of machine in the mechanized harvest, which, in most cases, is superior to defoliation caused by manual harvest, can reduce the productivity of the next year, because the

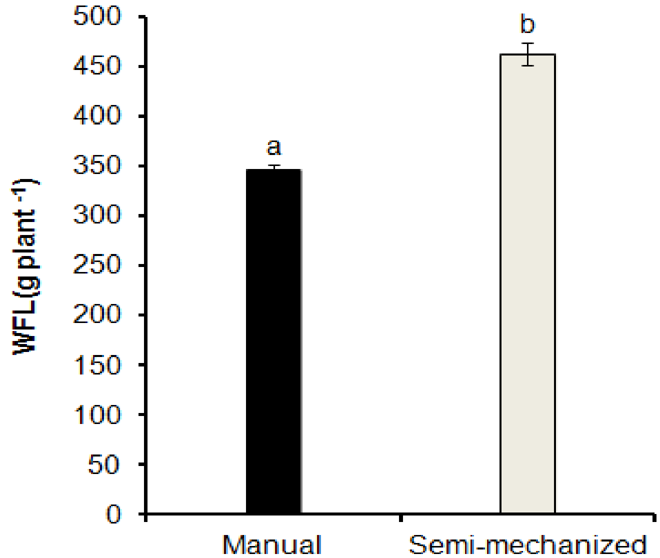


**Figure 4.** Weight of fallen leaves (WFL) in manual and semi-mechanical harvest of coffee fruit. Means followed by the same letter do not differ by the Tukey test at 5% probability. Each value represents the mean ± S.E. (n=6).

plant will use its reserves for vegetation restoration, which can result in stress and reduce its longevity. In turn, Cassia et al. (2013) describe that the damage caused to plants by mechanical harvest showed defoliation values within the acceptable.

The fruits are preferred drains to photoassimilates, during the reproductive period, with a high degree of dependence on plant nutritional status and functional relationship between leaf and fruit, due to the high demand for nutrients (Laviola et al., 2009; Partelli et al., 2014), so higher defoliation can influence the productivity of the next year. Silva et al. (2010) observed that manual harvest drops more leaves in sites of higher productivity, resulting in reduced productivity in coffee crop in subsequent years, because of the increased defoliation in plants with higher productivity and subsequent reduction in the use of photosynthetically active radiation.

However, defoliation needs to be studied and evaluated carefully because weather events will affect leaf recomposition of the coffee plant, such as water availability, temperature, plant nutritional status, pests and diseases, among others. Thus, it is possible that up to 30% defoliation does not significantly compromise the productivity of coffee plantations, especially in more densely populated crops, where the leaf/fruit ratio is usually higher.

When the plants have many green fruit, there is a tendency for the operator or to increase the machine vibration to also remove the greens and/or increase the harvest time in each plant, which may contribute to increasing defoliation. Oliveira et al. (2007) observed increased defoliation with the use of a mechanical harvester for harvest and repass, and noticed that the increased defoliation contributed to the reduction of the next crop productivity. However, the authors argue that increased defoliation may have been aggravated by the occurrence of rust (*Hemileia vastatrix*).

Thus, the use of semi-mechanized harvest with aid of portable mechanical coffee harvester requires knowledge and management suitable to increase harvest efficiency and reduce damage to plants and hence the next crop productivity. As noted by Oliveira et al. (2007), the control of rust is needed to reduce the defoliation levels. Furthermore, there are products available that can accelerate or retard the ethylene synthesis (Silva et al., 2006; Dias et al., 2014) and thus increase the ripe fruit percentage and consequently the efficiency of the semi-mechanized method. Yet, the specifications for the regulation of the portable mechanical coffee harvester are critical to compose whole system efficiency (Souza et al., 2006).

It is noteworthy that, currently, several types of pruning have been used in coffee crop, with the goal of plants adapting and renewing and in some cases concentrate production in a given year (as a system termed “safra zero”) due to shortages and cost of labor in coffee regions. Thus, the higher defoliation caused by semi-mechanized method is not a limiting factor if, after harvest, some type of pruning takes place, for example, the type termed "esqueletamento" (cutting of plagiotropic

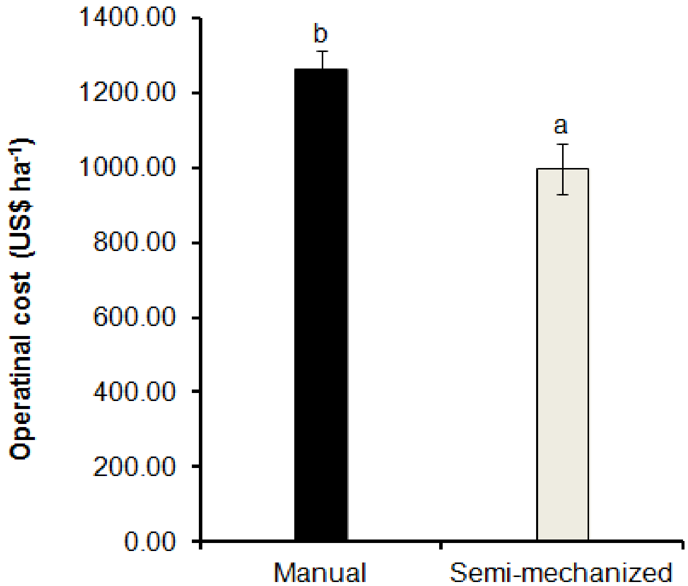


**Figure 5.** Operational cost in manual and semi-mechanical harvest of coffee fruit. Means followed by the same letter do not differ by the Tukey test at 5% probability. Each value represents the mean ± S.E. (n=6).

branches from 20 cm from the orthotropic branch).

In this study, the semi-mechanized showed higher defoliation, however, it showed similar value of TS to manual harvest method, but with a tendency to be higher in the first, if the sampling period is longer. However, even with higher TS, the repass seems to be the operation that contributed the most to reduce the net yield of the semi-mechanized harvest (Barbosa et al., 2005) and increased harvest cost. These factors can be mitigated by increased maturation uniformity of fruits and/or increased percentage of ripe fruit in the field.

Regarding the harvest operational cost, the semi-mechanized method showed a cost reduction of *ca.* 27% (Figure 5), close to the values obtained by Barbosa et al. (2005), which observed a reduction from 57 to 74% for a yield with plants of 1.3 m height, 6.27% green fruit, in the south Minas Gerais, Brazil. However, the authors observed large variation in the operation cost for various crops conditions and machine regulation, with a cost reduction for the semi-mechanized harvest of 28% on average.

Besides being useful for harvest, it is also worth mentioning the feasibility of the portable mechanical coffee harvester, it can be transformed or adapted to function as a weed control equipment or pruning machine, thinning the costs and expanding its use in crop management, reducing the problem of labor, which would help in reducing the overall cost of management of coffee plantations.

The data presented in this study reinforces the use of semi-mechanized harvest with the aid of portable mechanical coffee harvester as a viable solution to the mountainous regions, since they allow a quicker and cheaper harvest. Other studies should be conducted, in long term, in order to verify the effect of continuous use of portable mechanical coffee harvester, on the crop productivity and longevity, for systems that do not use systematic pruning.

## Conclusion

The semi-mechanized harvest method with the aid of portable mechanical coffee harvester provided the highest yield at harvest and the lowest operating costs. The semi-mechanized harvest showed the highest defoliation.

## Conflict of Interest

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

## ACKNOWLEDGMENTS

The authors wish to thank Fundação Carlos Chagas de Apoio à Pesquisa do Estado do Rio de Janeiro (FAPERJ) for financial support and Fazenda Panorama for supplying the experimental area.

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academicJournals

Vol. 10(28), pp. 2731-2737, 9 July, 2015
DOI: 10.5897/AJAR2015.9524
Article Number: 986882153974
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

*Full Length Research Paper*

# Carcass quality audit - A strategy to improve beef sector in Ethiopia

**YesihakYusuf Mummed* and Edward Cottington Webb**

Department of Animal and Wildlife Science, University of Pretoria, Private bag X20, Hatfield, Pretoria 0028, South Africa.

Received 20 January, 2015; Accepted 20 May, 2015

**Ethiopia has the largest cattle population in Africa. However, the potential of the sector has not fully utilized. Average carcass weight of cattle, per capita meat consumption and annual volume of meat produced were very low. Experience from developed countries indicates that implementing carcass quality audit would aid to identify quality problems, develop strategies and establish an educational plan to improve carcass quality. The audit has helped in benchmarking carcass quality parameters to quantify the progress of the sector at intervals of time. The purpose of this paper is to develop strategy on beef carcass quality audit and indicates the possibilities of implementing it at beef export abattoirs in Ethiopia.**

**Key words:** Beef, carcass, audit, Ethiopia.

## INTRODUCTION

### Constraints and opportunities of beef export in Ethiopia

Livestock plays an important role in the agriculture of Ethiopia. It contributes 15 to17% of Gross Domestic Product (GDP) and 35 to 49% of agricultural GDP, and 37 to 87% of the household incomes (CSA, 2008). Ethiopia has 53.4 million cattle, 25.51 million sheep and 22.79 million goats (CSA, 2010/2011). The potential of these resources have not been fully utilized. Average beef carcass weight at Ethiopian abattoirs was 135 kg (Mummed and Webb, 2014). Ethiopians consumed about 8 kg of meat per capita annually, which is far less than what is consumed in developing countries (Sebsibe, 2008).

Middle East and North African countries are potential markets for the export of livestock and meat product (NEPAD–CAADP, 2005). The annual demand of meat by these regions was estimated about 316,846 tones. However, Ethiopia exported about 16, 877 MT of meat to this region in 2010/2011 (SPS-LMM, 2011). Geographical proximity of the country to Egypt and the Gulf region compared to major meat suppliers to the region such as Brazil, India, Pakistan, Australia and New Zealand is one of the advantages (SPS and LMMP, 2010). In Ethiopia, the policy developed by the government to increase meat export has provided good opportunities for the development of the sector. The increases in the income of Ethiopians and growth of population have created a big demand for meat production. The rapid growth in the meat demand in the Gulf region is an opportunity that should not be missed (Hutcheson, 2006). Factors

*Corresponding author. E-mail: yesihakyus@gmail.com, Tel: +27617500867.

hindering Ethiopia’s competitiveness in the region are unreliable continuous supply and low quality of meat (Farmer, 2010). Dark cutting, improper handling of the product, poor sanitation, careless packing, poor management during transport, lack of continuous supply and unresponsive business communications were some of the reported problems of meat in Ethiopia (Anon, 2006). Feedback from importing countries revealed that they are not satisfied with the quality of meat imported from Ethiopia (Farmer, 2010).

Ethiopia has imported significant amounts of meat from the United States of America (USA), United Arab Emirates (UAE), Italy, the Netherlands, China and South Africa. One of the main reasons given by meat importing firms in Ethiopia for importing meat from other countries was the unavailability of higher quality meat in domestic markets (SPS and LMMP, 2010). This indicated that the quality of meat produced in Ethiopia does not satisfy the quality requirements of domestic consumers.

From 2005-2011, sanitary and phytosanitary standards and livestock and meat marketing program has worked in close collaboration with public and private institutions to increase meat and live animal exports from Ethiopia. During this period, meat exports were doubled (from 7,917 MT to 16,877 MT), while official live animal exports were tripled (from 163,375 to 472,045 head). The program has assisted the public and private sectors to achieve their objectives by providing technical support to strengthen animal health and SPS certification systems. The program has helped in upgrading skills of individuals and sectors involved in animal feeding, processing, marketing and export of meat and live animals (SPS-LMM, 2011). The foundation which was laid to develop the sector by the SPS-LMM program needs to be sustained in the future to further improve the meat industry. One way of assuring quality, consistency and competitiveness of the beef industry is establishing export beef carcass quality audit (EBCQA) in Ethiopia. By establishing EBCQA, the quality and yield of carcass will be monitored at interval of time and problems will be minimized to improve the sector further. For the purpose, export abattoirs are the ideal center for the action as they have relatively better facilities and recording practice compared to the local abattoirs (Mummed and Webb 2014).

## National beef quality audit in developed countries

Beef quality audit was created in different countries to improve the quality of beef. The aim of the audit in USA was to identify and measure quality problems and to establish an educational plan to address the problems identified. In USA audits were conducted in 1991, 1995, 1999, 2000, 2005 and 2010/2011 (BQA, 2003; Savell et al., 2011). Moreover, the audit was aimed to measure quality defects, which could be managed primarily through the efforts of cattle producers. It developed benchmarking quality parameters to measure the progress of the sector at different interval of time. The NBQA supported the development of strategies to reduce the incidence of defects. The ultimate objective of the NBQA is to enhance the quality and safety of beef while increasing the profitability of a country from beef and cattle industry (NBQA, 2010/2011). For this purpose, quantitative data was collected from several abattoirs on the slaughter floor and in the cooler for analysis. The goal of the National Beef Quality Audit (NBQA) in Canada is to continually improve the value of Canadian beef carcasses by delivering a consistent high quality, safe product to consumers domestically and around the world (NBQA, 2010/2011). In Canada, the audit was conducted in 1995, 1998/1999 and 2010/2011 (NBQA, 2010/2011). In Romania national grading of quality of beef carcass and veal was conducted in 2008 (Petroman et al., 2009). The aim of the grading was to classify carcass and evaluate yield, and report the result of the classification to Romanian Commission of Pork, Beef and Sheep Carcasses Grading (Petroman et al., 2009). The report was used to make the correct payment to beef breeders, according to carcass weight and quality, and standardization, the development of common language in the international meat trade. In Italy, effectiveness of carcass data collection in cattle slaughterhouse was evaluated in 2007 (Lazzaroni and Biagini, 2009). Data on carcass was collected to verify the effectiveness of the application of the UE carcass classification in a cattle slaughterhouse. In this work data were collected and analysed to highlight the critical point and to improve the recording performance of the technical staff and to avoid the possibilities of making mistakes in registration (Lazzaroni and Biagini, 2009).

Different institution took the responsibilities of the auditing in different countries. In USA, Colorado State University, Oklahoma State University and Texas A&M University participated in collecting data for the auditing. Between May and November 2000 thirty packing plants were audited. The survey teams assessed hide condition from 43,415 cattle, incidence of bruises from 43,595 carcasses, offal and carcass condemnation from 8,588 cattle, and carcass quality and yield information from 9,396 carcasses. The data was collected once in the spring/summer and once in the fall/winter from 50% of each lot on the slaughter floor and 10% in the cooler during a single day’s production (one or two shifts, as appropriate). Based on the collected data, carcasses were evaluated for coat color, breed/origin of cattle, brands, mud/manure, means of identification of cattle, sex, bruises, dentition, offal and carcass condemnation, carcass quality and yield information (Boleman et al., 1995; McKenna et al., 2002). In Romania and Italy, data was collected on categories of cattle slaughtered, degree of conformation, degree of fatness, traceability of slaughter animals (breed, sex, live weight and carcass weight), carcass defects and condemnations (Lazzaroni and Biagini, 2009; Petroman et al., 2009). Based on the audit made different solution were sought as per the aim of the audit made in different countries.

## Carcass grading/ Classification

Evaluations of carcass and meat quality are important practices in meat marketing at national and international level (Lazzaroni, 2007). Interest and questions about quality of meat are on the rise due to heightened awareness about the marketing of beef and meat, from procurement and processing to consumer acceptance (Lazzaroni, 2007). Producers are now beginning to receive information about quality of meat they produce. New marketing structures such as vertical integration and value based marketing provided direct financial rewards to cow-calf producers who offer more desirable carcasses (Drake, 2004). Carcass grading and classification system improve communication between producers, traders and consumers. These systems help to develop clearer market signals from the consumer to the producer, act as a catalyst for breed and national herd/flock improvement, act as a framework for the development of national price reporting schemes, assist producers to market their stock more effectively, improve efficiency in transactions, promote retail sale by the marking or labeling of grading information on meat and facilitate the development of any export markets (Strydom, 2011). Because of these benefits carcass grading and classification systems are continuously being developed to describe the quality and yield of a carcass.

On the global scale there are two predominant grading schemes. These are the USDA grading schemes and EU classification scheme. USDA grading scheme evaluate carcasses based on class of animals (steer bullock, bull, heifer, cow), maturity (meat color and texture), quality grade (Prime, choice, Select, Standard, Commercial, Utility, Cutter, Canner) and yield grade. Yield grade estimates amount of closely trimmed retailed cut of meat that the carcass is likely to produce. The yield grades range form 1 to 5, with 1 the highest yielding 5 the lowest yielding. EU classification system (SEUROP) evaluates carcasses based on class (calf, young bull, bull, steer, heifer, cow), conformation grade (six levels) and fat grade (five levels; Fisher, 2007). Classification is a set of descriptive terms describing features of the carcass that are useful as guidelines to those involved in the production, trading and consumption of carcasses and meat, whereas grading is the placing of different values on carcasses for pricing purposes, depending on the market and requirements of traders and consumers. Criteria used in carcass grading systems rank carcasses fairly accurately according to expected eating experience of muscles. Criteria used in carcass classification systems give limited descriptions of the quality related characteristics of the carcass (Strydom, 2011). In UK beef carcass classification scheme was launched in 1972 using carcass weight, conformation, category and age (AHDB Industry Consulting, 2008). Similar schemes were developed in other European countries with Germany adopting a compulsory scheme in 1968 with 4×3 conformation/fatness grid. Classification in Ireland and France was similar in nature (AHDB Industry Consulting, 2008). The British and rest of Europe's classification systems evolved over many years and were combined into a single system (EUROP) in 1981 (AHDB Industry Consulting, 2008) with the main objective to describe carcasses for those involved in slaughtering, cutting, distribution and retailing according to terms relevant to trading. Adoption of the EUROP system within the EU enabled those involved in the production, slaughtering, cutting, distribution and retailing of meat to describe carcasses in terms that others would understand and that were of commercial relevance in trading. In addition to market reporting standardised description also provided a base for administration of support payments. While the support payment role has now ceased the market reporting function system remains central to beef marketing in Europe (Polkinghorne and Thompson, 2010).

The grading method practiced in different countries varies depending on the objectives of the system and on the degree of uniformity that exists among types and species of animals. The USA and Australia use a grading system based on marbling, age and sex of slaughter animals. In South Africa, carcass classification is used based on external fat covering, conformation and age of the animal. In Australia the meat grading system (AUS-MEAT) and Meat Standard Australia (MSA) are the only systems using pre-slaughter criteria, while the other grading systems perform measurements on the slaughter floor. Chiller assessments are used by all but the SEUROP and South African (SA) systems. The MSA system performs post-chiller assessments. Conformation, shape or rib eye area (REA), some form of fat measurement, carcass weight and sex are common criteria for all systems and are recorded on the slaughter floor and/or during chiller assessment (Table 1).

In South Africa beef description systems have evolved over a long period (Strydom, 2011). A carcass grading system was used from 1932 to 1985 (Strydom, 2011). The grading system was replaced by a carcass classification system in 1992 (Strydom, 2011). The change in the system was aimed to describe carcasses in more objective terms, which allowed buyers to select their ideal article for a purpose rather than impose a universal hierarchical grade structure (Anon, 2006b). The South African system classifies carcasses into four age categories derived from dentition denoted as A (no permanent incisors), AB (1–2 permanent incisors), B (1–6 permanent incisors) and C (greater than six permanent incisors). Bulls in age category B or C are noted and

**Table 1.** Principal component of selected beef classification and grading schemes in selected countries around the world (Adopted from Strydom, 2011).

| Scheme | Canada | SEUROP | Japan | Korea | S. Africa | USDA | Aust-Meat | Meat standard |
|---|---|---|---|---|---|---|---|---|
| Grade Unite | Carcass | Carcass | Carcass | Carcass | Carcass | Carcass | Carcass | Cut |
| Classification | -- | Yes | -- | -- | Yes | -- | Yes | -- |
| Quality grade | Yes | -- | Yes | Yes | -- | Yes | -- | Yes |
| Yield grade | Yes | -- | Yes | Yes | -- | Yes | -- | -- |
| Pre-slaughter | -- | -- | -- | -- | -- | -- | Grain fed | Bosindicus % HGP implant |
| Slaughter floor | Carcass wt<br>Sex<br>Conformation | Carcass wt<br>Sex<br>Fat cover<br>Conformation | Carcasswt<br>Sex | Carcasswt<br>Sex | Carcass wt<br>Dentition<br>Fat cover Conformation<br>Sex | Hot Carcass wt<br>Sex | Carcass wt<br>Sex<br>Dentition<br>Butt shape<br>P8 fat | Carcass wt<br>Sex<br>Electric stimulation Hang |
| Chiller | Marbling score<br>Meat core<br>Meat texture<br>Fat color<br>Fat thickness<br>Skeletal development | -- | Marbling<br>Meat color<br>Meat brightness<br>Fat color<br>Fat lust<br>Fat texture<br>Fat firm<br>EMA<br>Rib thickness<br>Fat thickness | Marbling score<br>Meat color<br>Fat color<br>Firmness<br>Meat texture<br>Lean maturity<br>EMA<br>Fat thickness | -- | Marbling<br>Ossification<br>Meat color<br>Meat texture<br>Rib fat<br>EMA<br>Kidney and perennial fat | Left cold Half Marbling<br>Meat color<br>Fat color | Marbling<br>Ossification<br>Meat color<br>Hump height<br>Ultimate pH |
| Post chiller | -- | -- | -- | -- | -- | -- | -- | Aging time<br>Cooking method |

denoted MD. Seven fat classes denoted as numerals from 0 (no visible fat) to 6 (excessively fat) are added to the age group and the combination applied as a colored roller marker or brand to carcasses after classification. Colors (purple for A, green for AB, brown for B, red for C and black for MD) represent the age in classification. Five numerical carcass conformation classes - 1 (very flat), 2 (flat), 3 (medium), 4 (round) and 5 (very round) are also designated together with three damage codes of 1 (slight), 2 (moderate) and 3 (serious) where applicable (Anon, 2006b).

USDA grading system was developed for cattle finished in feedlots. Marbling and age of cattle are the major parameters used to determine quality of carcass. Moreover, this grading system was mainly developed to evaluate cattle up to 24 months of age. Steers and heifers are the only type of animals considered for top quality beef in the system (ZoBell et al., 2005).

Most Asian and European countries use classification systems instead of grading system. Middle East countries are the potential market for meat produced in Ethiopia (NEPAD–CAADP, 2005). Hence, the development of a carcass classification system instead of grading by the Ethiopian standard agency was the appropriate choice as the potential export market for the country is Middle East countries. Ethiopia has developed a beef carcass classification system in 2012, which is a modification of the SEUROP classification system (ES, 2012). The Ethiopian

**Table 2.** Characteristics and description of Ethiopian beef classification (ES) system (ES, 2012).

| **Conformation** | **Grade** |
|---|---|
| Carcasses with convex profiles and very well developed muscle | 1 |
| Carcasses with straight profiles and good muscle development | 2 |
| Carcasses with concave profiles and moderate muscle development | 3 |
| **Fat** | **Grade** |
| Carcasses with small or no fat coverage | 1 |
| Carcasses with fat visible on the whole body exception the hind leg and shoulder | 2 |
| Whole carcasses covered with fat and fat deposited in the thoracic cavity | 3 |
| **Descriptions** | **Categories** |
| Carcass of young bull or heifers that weight less than 70 kg | JB |
| Carcasses of grown up bulls (cartilage of the spine up to four thoracic vertebras show no sign of ossification and from fifth to ninth show sign of ossification; discs of inter-vertebral of sacral vertebrae show sign of ossification) | JM |
| Carcasses of mature intact bulls | M |
| Carcasses of castrated bulls | O |
| Carcasses of heifers | JF |
| Carcasses of cows | F |

classification system structured per animal categories, conformation and fat grade as shown in Table 2. Carcass grading schemes differ all over the world in terms of specific technique, yet most of them include some form of assessment of both fatness and muscle development (Strydom and Smith, 2005). Carcass quality is mainly determined by age, sex, conformation and fat grades (Lazzaroni and Biagini, 2009). Conformation is defined as thickness of the muscle, intermuscular fat and subcutaneous fat relative to the dimensions of the skeleton (De Boer et al., 1974). Adequate fat cover must be present to produce corresponding marbling that determines quality of the product (Mummed and Webb, 2014). The animal category in Ethiopian beef carcass classification system represent the age, gender and intactness/castration of the bulls (Table 2). This can indicate that the Ethiopian beef carcass classification system contains all parameter required to classify carcasses.

## Establishing beef carcass quality audit in Ethiopia

A carcass quality audit is one way of identifying and measuring carcasses quality problems. It is a base to establish an educational plan to address the problems identified. Quality defects, which can be managed primarily through the efforts of cattle producers, will be identified. Benchmarking quality parameters will be set to quantify the progress of the sector at specified intervals or years. A strategy to reduce the incidence of defects will be developed. The objective of the audit that will be established in Ethiopia will be to enhance the quality of beef while increasing the profitability of the Ethiopian beef industry. Quality problems in beef carcasses will be ranked and educational programs will be arranged to address these challenges.

The carcass classification system developed by Ethiopian Standard Agency in 2012 is a good opportunity to implement export beef carcass quality audit (EBCQA) at export abattoirs in Ethiopia. The reason for the implementation of the program on export abattoirs is due to the better facilities and the good practice of recording information in these abattoirs compared to the local abattoirs (Mummed and Webb, 2015). Moreover, recently MOA has developed a sector solely focusing on the export of livestock and livestock products. This sector will play a coordinating role in the execution of EBCA in Ethiopia. The audit will be conducted at two years intervals as the number of export abattoirs and the numbers of animals slaughtered per day are small compared to USA and Canada. Experience from the countries conducted the audit shows that collection of data can be accomplished by universities. The activities will be conducted for few numbers of days (5-10) per year. The universities should publish reports on the status of the carcass production and quality every other year. These reports will identify major problems associated with carcass yield and quality. The concerned body (beef export sector of MOA) should prioritize these problems and seek solutions through the extension service program. A strategy will be developed to reduce the proportion of quality problems, defects, causes of condemnation of carcasses and organs in short and long term program. For institutes involved in the auditing activity, it is one way of serving the industry beyond their academic exercise. For these institutes, it will be an excellent opportunity to get retrospective and prospective data for research purposes. Most of the data required for the audit involves information recorded already in the abattoirs. Abattoir personnel usually record information on traceability of slaughtered animals such as breed (source of purchase), sex, live weight and carcass (weight. Federal Veterinary inspectors are well established in recording carcass defects and condemnations. Additional tasks expected from institutes involved in the auditing activity will be collecting information on classification of the carcasses (category, conformations and fat grade). This work will be done parallel to collecting recorded information at the abattoirs. One week training for personnel involved in the data collection on classification system will be sufficient to avoid subjective difference between technicians. Involving universities in different regional states will further minimize the cost of transport of researchers and materials. Gijiga University, Haramaya University, Hawassa University, Bahrdar University and Mekelle University can conduct audits at export abattoirs in Solmali, Oromiya, Southern People National and Nationalities (SPNN), Amhara and Tigray regional states, respectively. However, institute like Haramaya University will take the responsibility of managing data, analyzing and writing the report because of the long experience in research and teaching activities. Data base management should be established at this center. Those data collected at interval of a year and used for reporting at specific years need be stored in this center. After long period of time, say ten years, these data at the management center will be used to develop long term strategy to solve quality problems. The out put of the activity should be publishable rather than a mere report to the concerned body and academic exercise. This will be an opportunity to monitor the status of beef production and constraints in Ethiopia so that every concerned body will aware of the situation. By developing export carcasses audit, the major yield and quality problems will be identified and profound solution will be found. The solution of these problems will be feedback to producers through extension education so that better quality beef and meat will be produced for export market. This will sustain the quality, consistency and competitiveness of the country in meat industry.

## CONCLUSION

The large cattle resources available in Ethiopia are not fully utilized. One of the major problems was inability to produce quality product for export and local consumption. Experience from developed countries show that establishing carcass quality audit will assist to identify problem and develop strategy to improve the sector. It is therefore suggested that establishing export beef carcass quality audit (EBCQA) in Ethiopia will be good opportunity to improve the sector, sustain the quality, consistency and competitiveness of the country in beef industry.

## Conflict of Interest

The authors declared that there is no conflict of interest.

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academicJournals

Vol. 10(28), pp. 2738-2747, 9 July, 2015
DOI: 10.5897/AJAR2014.9360
Article Number:9B1EBD653970
ISSN 1991-637X

http://www.academicjournals.org/AJAR

**African Journal of Agricultural Research**

*Full Length Research Paper*

# Willingness to pay for rainfall risk insurance by smallholder farmers in Central Rift Valley of Ethiopia: The case of Dugda and Mieso Districts

**Hiwot Teshome[1]* and Ayalneh Bogale[2]**

[1]Ethiopian Institute of Agricultural Research (EIAR) National Fishery and Aquatic Life Research Center, Sebeta, Ethiopia.
[2]University of KwaZulu-Natal School of Agriculture, Earth and Environmental Sciences Pietermaritzburg, South Africa.

Received 21 November, 2014; Accepted 18 June, 2015

**Current climate variability is already imposing significant challenge. Therefore, farmers have faced income variability in almost every production season. Problems associated with dependence on rain fed agriculture are common in Ethiopia. Smallholder farmers' vulnerability from such income variability is also common. Over the years, a range of risk management strategies have been used to reduce, or to assist farmers to absorb, some of these risks. Since insurance is potentially an important instrument to transfer part of the risk, the study tries to assess small holder farmer's willingness to pay for the rainfall risk insurance and examine factors that affect farmer's willingness to pay amount. The sample size 161 households using closed ended value elicitation format followed by open ended follow up questions. Logit model was used to estimate the mean willingness to pay in the close ended format in addition with Tobit model to examine factors that affect willingness to pay as well as intensity of payment. Six potential explanatory variables income, ownership of radio, off-farm income, age, number of livestock owning and availability of public and private gifts have negative and/or positive effect on WTP.**

**Key words:** Rainfall risk insurance, contingent valuation method, willingness to pay, smallholder farmers.

## INTRODUCTION

Agricultural producers around the world are exposed to a variety of income uncertainties, both market related, such as price variations, as well as non-market related, such as unstable weather patterns. It is well known that such uncertainties induce substantial income risks, and these can be particularly detrimental to poor producers in developing countries (Sarris, 2002). Due to the scope and diversity of such risks, formal insurance markets are scarce in such settings, and farmers employ relatively sophisticated methods to offset the risks they face (Clarke and Dercon, 2009). Hence, various challenges due to climate variability recognize that adaptation is not

*Corresponding author. E-mail: Hiwothiwi2000@gmail.com.

an option but a necessity (Thornton et al., 2006) this is because climate change is expected to have adverse affect on agricultural production. Which remains to be the main source of income for most countries (Bryan et al., 2009), farmers have developed several ways for dealing with the various risks they face. According to Stern (2007), adaptation to climate change and variability will be crucial in reducing vulnerability and is the only way to cope with the impacts that are inevitable over the next few decades.

Over the years, a range of risk management strategies have been used to reduce, or to assist farmers to absorb, some risks. These strategies include on-farm measures such as diversification or selecting less risky production methods, as well as strategies for sharing risk with others. Risk management strategies in which risks have shared with others include, among others, farm financing, share-cropping, price pooling arrangements, forward contracting of farm products, and hedge on future markets.

Hence, insurance is potentially an important instrument to transfer part of the risks (Anderson, 2001) the following two basic reasons can be raised as an advantage of preferring weather-based insurance other than crop insurance. First, weather index insurance contracts require less monitoring to control adverse selection and moral hazard (Hazell, 1999). According to (Stefan et al., 2012), risk-sharing and index insurances are complementary, with increase access to one driving up demand for the other, so that providing index insurance may crowd in informal risk-sharing. Even though, index insurance is an unfamiliar and complex product, providing training to farmers about index insurance has been shown to be important in encouraging take-up (Gine et al., 2012). It has been believed that training has a power to increases awareness as a result demand has also increased.

Similarly Stefan et al. (2012), also supports this idea but type of training provided to leaders of indigenous groups has important implications for demand for insurance, the strengthening mechanisms to manage basis risk makes index insurance more attractive to small-holder farmers. The second reason is weather-based insurance can prevent the problem of adverse selection (that is, since farmers know more about their risks than the insurer, the low-risk farmers may pick out, leaving the insurer with only high-risk customers) and moral hazards (that is, when farmers' behaviors can influence the extent of damage that qualifies for insurance payouts) (Linnerooth-Bayer and Hochrainer-Stigler, 2014).

However, insurances are growing rapidly in the developing world, as part of this growth; innovative new products allow individual smallholder farmers to hedge the risks, for instance, agricultural risks such as drought, disease and commodity price fluctuations (World Bank, 2005). These financial innovations hold significant promise for rural households. According to (Bezabih et al., 2011) crop riskiness at a farm level is highly responsive to rainfall variability and that the choice of high risk-high return crops is hampered by weather uncertainty. Shocks to agricultural income, such as a drought-induced harvest failure, generate movements in consumption for households who are not perfectly insured and at the extreme, may lead to famine or death.

Consequently, the variability is imposing significant challenge (Abera and Manfred, 2009) declare that rainfall has emerged as an important factor influencing household food security. Water and energy supply, poverty reduction and sustainable development efforts, as well as by causing natural resource degradation and natural disasters. In response, the national adaptation program of action (NAPA) for Ethiopia has been prepared, basic approach to NAPA preparation was along with the sustainable development goals and objective of the country where it has recognized necessity of addressing environmental issues and natural resource management with the participation of stakeholders (MoWR, 2007). However, an issue that was not yet addressed is whether there is demand as well as willingness to contribute by smallholder farmers for the new rainfall risk insurance. Therefore, the objectives of the study a) assess current risk management strategies practiced by the smallholder farmers b) identify willingness to pay as well as the extent of payment by the smallholder farmers.

## MATERIALS AND METHODS

### Study area sampling techniques

This method was originally designed to elicit the consumers' willingness to pay for a product that is not yet on the market, is now being widely used even for marketed good that have a substantial impact on the welfare of the society. After designing the draft questionnaire, pre-test was conducted with randomly selected sample households. An openly ended question was used for the elicitation of the respondents' maximum amount they are willing to pay for the insurance service per hectare. Pre-test was used due to make some modifications in the designed questionnaire and to obtain starting bid values. Based on this elicitation some values were selected as the starting bid values for the survey questionnaire (Figure 1).

A multi-stage sampling technique was used to select 161 sample households. In the first step of the sampling, out of the *districts* in the Central Rift Valley that have almost similar climate condition, "*Mieso*" and "*Dugd*a" *districts* were purposively selected because these areas are most drought prone areas. In the second stage, out of the 36 Peasant Associations (Pas) in "*Dugda*",4 PAs were selected randomly and of 36 PAs in "*Mieso*" *district,* 3 PAs were selected. In the third stage, the total numbers of households in each PAs were listed and finally a total numbers of 161 sample households were selected based on the proportion of the total number of households in each Pas. Then the bid values were randomly distributed to each questionnaire (161) and interviewed.

The respondents were asked whether they are willing to pay for a given amount or not, if the respondent says yes or no; finally the single bounded dichotomous choice question is followed up by an

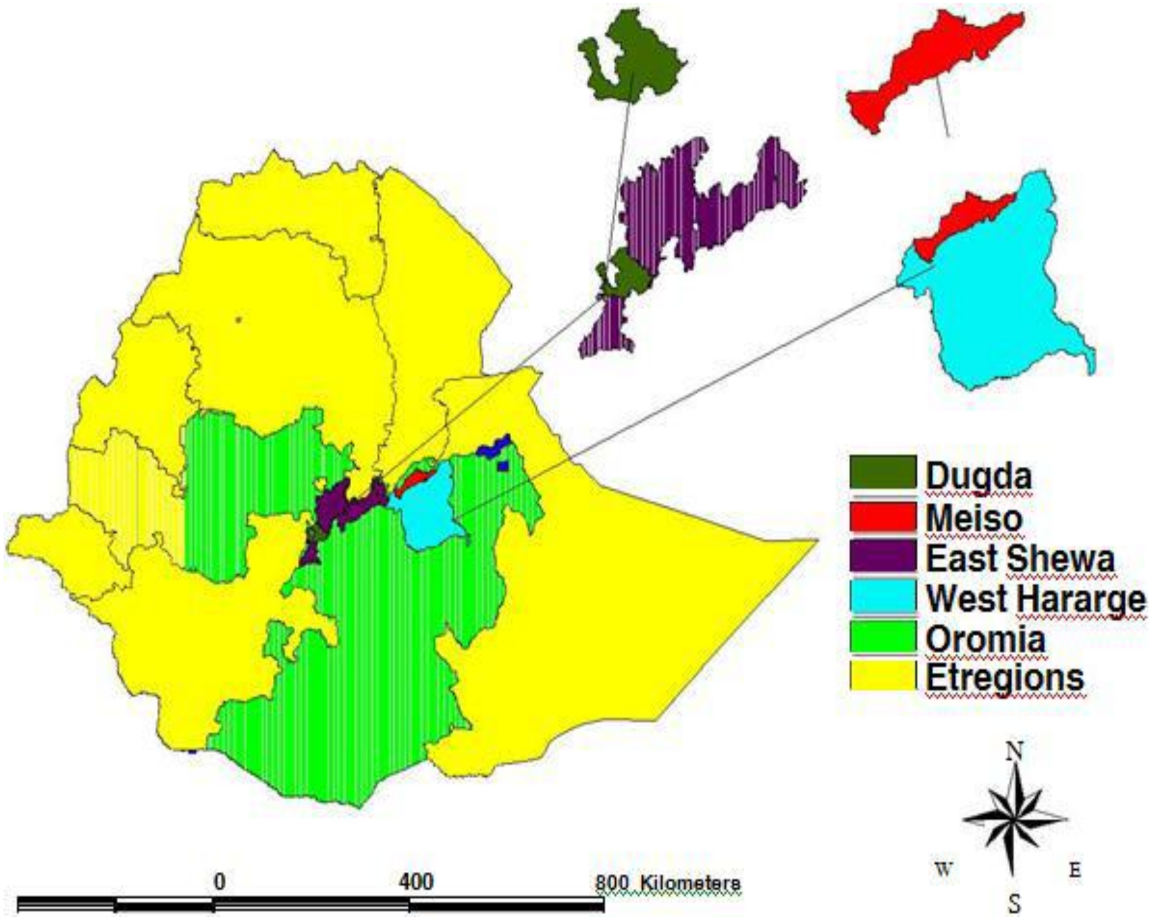


**Figure 1.** Location of the study area.

open-ended follow-up question. The use of an open-ended questionnaire is justified by its advantage indirectly eliciting the maximum willingness to pay and to avoid biases.

### Method of WTP data collection

Willingness to pay is defined as the amount that must be taken away from household's income. The willingness data is collected through contingent valuation method (CVM), this method is also suited to solicit consumers' willingness to pay for a product that is not yet on the market. CVM is now increasingly used in developing countries (Alberini and Cooper, 2000). In this method, the researcher creates a hypothetical market in a non-market or new good. The values which are generated through this hypothetical market are treated as estimates of the value of new good. After designing the draft questionnaire pre test was conducted with 26 randomly selected sample households. An open ended question was used for the elicitation of the respondents' maximum amount they are willing to pay for the insurance service per hectare. This is due to make some modifications in the designed questionnaire of the survey and to obtain starting bid values. Based on this elicitation some values were selected as the starting bid values for the survey questionnaire. The bid values were distributed randomly through 161 sample households and the respondents were asked are you willing to pay this amount if the respondent says yes or no, finally the single bounded dichotomous choice question is followed up by an open-ended follow up question.

### Data analysis

The data that had been collected through contingent valuation method has been analyzed using both descriptive statistics and econometric model. Descriptive statistics such as mean, percentage, standard deviation and frequency of appearance was used, whereas, on the econometric approach adopted Probit and Tobit models. The dependent variables are willingness to pay; dummy variable (yes or no) and the maximum amount of money the willing respondent are willing to pay.

### Model specification

Tobit model was commonly known as censored normal regression model (Greene, 2003). It assumes that many variables have a lower or upper limit that was known as a threshold value and take on this limiting value for a large number of respondents. For the remaining sample respondents, the variable takes on a wide range of values above the limit. The explanatory variables in the model may influence both the probability of limit responses and the size of non-limit. The two parts correspond to the classical regression for the non limit (continuous) observations and the relevant probabilities for the limit (zero) observations, respectively (Table 1). Based on the above behavior of the model, Tobit analysis is appropriate for this study and the formula for the Tobit model is described as follows; following Long (1997), the structural equation of Tobit model

**Table 1.** Variables and their measurement included in the mode.

| Variable | Code | Type of variable | Definition and Measurement |
|---|---|---|---|
| Age of the household | AGE | Continuous | Age of household head in years |
| Sex of the household | SEHH | Dummy | Sex of household head 1, if male 0, otherwise |
| Marital status | MRST | Discrete | Marital status 1, married 2, single 3, divorced |
| Location of the study area | NAWO | Dummy | Location of the study area 1, if in Dugda 0, in mieso |
| Family size | FSIZE | Continuous | Number of family members |
| Income from crop | FINC | Continuous | Total annual income of the households from crops in Birr |
| Education of the household | EDUC | Dummy | Education status of HHH, 1 if literate 0, otherwise |
| Off-farm income | OFINC | Continuous | Total off-farm income measured in Birr |
| Initial Bid value | BID | Discrete | Initial bid value offered in Birr per hectare |
| Maximum willingness to pay | MWTP | Continuous | Maximum WTP in Birr per hectare |
| Credit constraint | CREDIT | Dummy | 1 if the household is highly credit constrained 0, otherwise |
| Extension service | EXTENTION | Dummy | 1 service user 0, otherwise |
| Livestock holding | TLU | Continuous | Measured in tropical unit |
| Dependency ratio | DEPR | Continuous | Measured in Birr |
| House type | HOUSE | Dummy | 1 if iron roofed 0, otherwise |
| Owning radio | RADIO | Dummy | 1 if owning radio 0, otherwise |
| Availability of public and private aids | PAPA | Dummy | 1 if household head has aid 0, otherwise |

censored from below can be expressed as:

$$y_i^* = x_i\, \beta_i + \varepsilon_i \tag{1}$$

Where, *Yi* = the observed dependent variable, in this case the maximum willingness to pay the respondent is willing to pay in Ethiopian Birr; *YI**= the latent variable which is not observable; Xi = vector of factor affecting willingness to pay.

The model parameters can be estimated by maximizing the Tobit likelihood function of the following form (Maddala, 1997):

$$L = \prod_{Y_i^*>0} \frac{1}{\sigma} f\left(\frac{Y_i - \beta_i X_i}{\sigma}\right) \prod_{Y_i^* \le 0} F\left(\frac{-\beta_i X_i}{\sigma}\right) \tag{2}$$

Where f and F are respectively, the density functions and cumulative distribution function of > 0 means the product over those *I* for which > 0, and < 0 means the product over those *I* for which < 0.

Maddala (1997), proposed the following techniques to decompose the effects of explanatory variables into the decision to pay and intensity effects. Thus, a change in X (explanatory variables) has two effects. It affects the conditional mean of Yi * in the positive part of the distribution, and it affects the probability that the observation will fall in that part of the distribution. Similar approach will be used in this study:

i. The marginal effect of an explanatory variable on the expected value of the dependent variable is:

$$\partial E\left(Y_i\right) / \partial X_i = F_{(Z)} \beta_i \tag{3}$$

Where, $\beta_i X_i / \delta$ is denoted by *z*, and *F* is a cumulative distribution.

ii. The change in the probability of willingness to pay as independent variable Xi changes is:

$$\partial F(Z) / \partial X_i = f(z)\, \beta / \delta \tag{4}$$

iii. The changes in the amount of money respondent are WTP with respect to a unit change in an explanatory variable among those who are willingness to pay are:

$$\frac{\partial E\left(\frac{Y_i}{Y_i^*} > 0\right)}{\partial X_i} = \beta_i \left[1 - Z \frac{f(z)}{F(z)} - \left(\frac{f(z)}{F(z)}\right)\right] \tag{5}$$

Where: F (z) = is the cumulative normal distribution of z; f (z) = is the value of the derivative of the normal curve at a given point (unit normal density); z = is the z-score for the area under normal curve; β = is a vector of Tobit Maximum Likelihood estimates and σ = is the standard error of the error term.

**Table 2.** The result from Logit model to calculate the mean WTP.

| Variables | Coefficient | St. d | t-value | p-value |
|---|---|---|---|---|
| CONSTANT | 3.993626 | 0.6619955 | 6.03 | 0.0000 |
| BID | -0.021873 | 0.0042091 | -5.20 | 0.0000 |

In the logit model of single bounded dichotomous format, are given initial bid value in which they may accept or reject. In the logit model the dependent variable is dummy variable. The purpose of the Logit model is to estimate the mean WTP. Following Gujarati (1999), the Logit model is expressed as follows:

$$\text{Logit}\left(P(X)\right) = \beta_\circ + \beta_i x_i + \varepsilon_i \quad (6)$$

Where: $P(X)$ = probability that a given household is willingness to pay; $\beta_\circ$ = Constant term; $\beta_i$ = regression coefficient to be estimated or Logit parameter; $x_i$ = initial bid value; $\varepsilon_i$ = error term of the Logit regression.

One of the main objectives of estimating an empirical WTP model based on the CV survey responses is to drive a central value or mean of the WTP distribution Hanemann et al. (1991). According to Gujarati (1999) both Probit and Logit models provide similar results thus, for comparative computational simplicity Logit model was used for the estimation. And the mean willingness is formulated as:

$$\mathrm{E(WTP)} = \frac{\ln\left(1+\exp(\beta_\circ)\right)}{\beta_1} \quad (7)$$

Where: $\beta_1$ = bid coefficient; $\beta_\circ$ = Constant term.

## RESULTS AND DISCUSSION

### Descriptive statistics results

Descriptive statistics such as mean, minimum and maximum values, range and standard deviations were used to describe the major factors explaining farmers' willingness to pay for rainfall risk insurance. In addition, mean difference for continuous variables and frequency of discrete variables were tested using t-test and chi-square test respectively by using (SPSS V-16).

### Perception of risk

Households in the study area perceive that they are exposed to a variety of substantial risks from different sources. Therefore, based on the results obtained from formal survey questionnaire, households define risk in three ways: year when rainfall delays, year when rainfall is inadequate, year when rainfall is high.

### Risk management strategies

In order to cope with sources of risks, rural households have developed various risk management strategies which only differ from place to place, and among the farmers. Therefore, sale of livestock in case of emergency is a major risk coping strategy practiced by farmers others, diversification use of improved technology, delay in a sale of crop products and intercropping were also strategies used by farmers which are listed according to their importance.

### Willingness to pay analysis

The total sample households were randomly distributed to the four initial bid values (50, 100, 150, 200), each value contains 41, 47, 36 and 37 respondents respectively. Out of the total sample respondents 17(27.2%) responded "no" to the initial bid value. The main reason farmers have refused to accept the service includes they could not afford it, and they did not trust the service very well. But the rest 144(72.8%) show their interest to contribute and gave a "yes" or "no" response to the initial bid value then follow-up values.

### Estimation of the mean WTP

The initial bid value was regressed with the dependent dummy variable, the result of the coefficients were showed in Table 2, and willingness to pay for the single bounded dichotomous format is as follows:

$$\mathrm{E(WTP)} = \frac{\ln\left(1+\exp(\beta_\circ)\right)}{\beta_1}$$

Where:

$\beta_1$ = 0.021873

$\beta_\circ$ = 3.993626

$$= \frac{\ln\left(1+\exp(3.993626)\right)}{0.021873} = 183.41$$

Thus, the mean willingness to pay calculated from the single bounded dichotomous format is 183.41 Ethiopian birr per hectare. However, the mean WTP is 129.93 Ethiopian birr per hectare from responses to the open-ended CV survey questions, which is lower than the mean value obtained from the closed-ended Logit model estimates. Thus, the result showed that the respondents were willingness to pay between the ranges of 129.93 to 183.41 Ethiopian Birr per hectare for the proposed rainfall

**Table 3.** Total willingness to pay and total revenue in Eth (Birr).

| Class bound. For WTP amount | Class mark for WTP amount | Sample District of HHs | | Total no of HHs | Total WTP in Ethiopian (Birr) | Sample HHs WTP at least that amount | | Total HHs WTP at least that amount | Total Revenue |
|---|---|---|---|---|---|---|---|---|---|
| | | N | % | | | N | % | | |
| 0-50 | 25 | 39 | 24.223 | 12,103.3 | 302,582 | 161 | 100 | 49,966 | 1,249,150 |
| 51-100 | 76 | 54 | 33.54 | 16,759.6 | 1,273,729 | 122 | 75.5 | 37,864 | 2,877,664 |
| 101-150 | 126 | 11 | 6.832 | 3,414.18 | 430,186 | 68 | 42.24 | 21,105 | 2,659,230 |
| 151-200 | 176 | 37 | 22.981 | 11,482.7 | 2,020,953 | 57 | 35.40 | 17,688 | 3,113,088 |
| 201-250 | 226 | 7 | 4.347 | 2,172.02 | 490,877 | 13 | 8.07 | 4032 | 911,232 |
| 251-300 | 276 | 7 | 4.347 | 2,172.02 | 599,478 | 11 | 6.83 | 3413 | 941,988 |
| 301-350 | 326 | 5 | 3.105 | 1551.44 | 505,771 | 6 | 3.73 | 1864 | 607,664 |
| 351-400 | 376 | 1 | 0.621 | 310.29 | 116,669 | 1 | 0.62 | 310 | 116,560 |
| Total | | 161 | 100 | 49,966 | 5,740,244 | | | | |

risk insurance service.

## Estimating total willingness to pay and total revenue

The total willingness to pay and total revenue at different prices that households in the seven PAs of the two districts ("*Dugda"* and "*Mieso"*) were willing to pay as computed. The sampled seven PAs namely, *(B/Gusaa, Odd Bokota, Jawe Bofo, S/wakalee, Huse mandhera, Chobi, Burimulu*) have a total of 3281 households with a total population of 49,966 households with a total population of 275,307 and the average family size of 5.86. Based on this information and the distribution of WTP by the respondents, it would be possible to estimate the expected total willingness to pay and total revenue for the study area. Table 3 provides the procedure and results of the analysis.

The first column shows the maximum willingness to pay interval, and the second is class for willingness to pay (the mid willingness to pay) of the first column. The third and the fourth columns show the number and the percentage of sample households whose willingness to pay amount falls within a given interval. The total number of households in two districts of the study area has multiplied by the proportion of sample households falling under each category to obtain the total number of households whose willingness to pay lies in each boundary (column fifth). And total willingness to pay (column sixth) has been obtained by multiplying the mid willingness to pay by total number of households willingness to pay. The total household of 49,966 in two districts of the study area was expected to pay ET birr 5,740,244 / year if every household insures one hectare of his/her land.

Therefore, the result shows that the average insurance premium payment was ET birr 114.88 / hectare/ household if the proposed insurance service has implemented. This result is almost similar with an average willingness to pay ET 129.93 / hectare/ household. A column seven and eight represents the number and the percentage of sample household willingness to pay at least the amount in each interval. Similarly, column nine shows total number of households willing to pay at least the amount in each interval and it falls when the mid willingness to pay rises (column ten). Total revenue has been obtained by multiplying the mid willingness to pay amount (column two) by the corresponding total number of households' willingness to pay at least that amount, (column nine).

## Derivation of aggregate demand

The aggregate demand has been derived from the above willingness to pay scenario (Table 3). Any point on the curve shows all the respondents that prefer the insurance service, but do not bid more than the corresponding value on the mid willingness axis. The demand curve is negatively sloped, indicating the fall of the demand for the insurance service as an insurance premium increases, like most other non-market goods other things remaining constant. The area under demand curve represents the gross value of

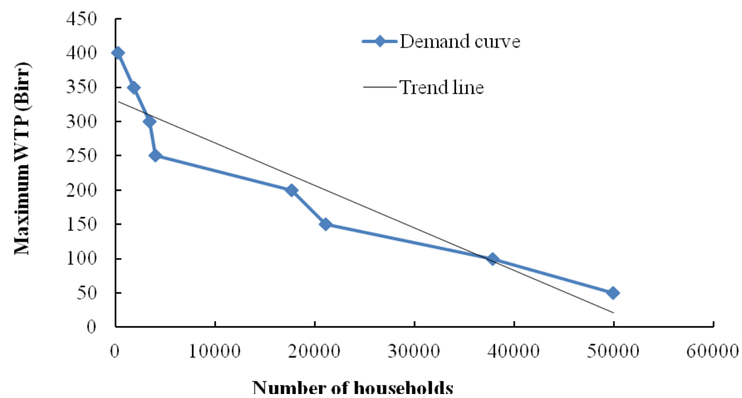


**Figure 2.** Estimated demand curve for rainfall risk insurance service.

**Table 4.** Maximum Likelihood estimates of the Tobit model.

| Variables | Estimated coefficient | Standard error | t-ratio |
|---|---|---|---|
| Constant | 3.70112 | 103.639 | 0.0357 |
| NAWO | -2.77379 | 18.8725 | -0.14697 |
| SEHH | 105.47 | 62.4742 | 1.68821 |
| AGE | -1.72656 | 0.71336 | -2.42032** |
| EDUC | 7.89432 | 15.9354 | 0.49539 |
| MRST | 22.2316 | 22.1423 | 1.00403 |
| FINC | 0.00556 | 0.00155 | 3.59743*** |
| OFINC | -0.01032 | 0.00487 | -2.12119** |
| DEPR | -0.66475 | 8.84637 | -0.07514 |
| FSIZE | 5.18168 | 3.7561 | 1.37954 |
| TLU | -5.01802 | 1.64755 | -3.04575*** |
| CREDIT | -9.49309 | 15.8865 | -0.59756 |
| EXTENTION | 24.4899 | 22.8428 | 1.0721 |
| PAPA | -49.3245 | 16.0296 | -3.0771*** |
| BID | 0.05866 | 0.12318 | 0.47623 |
| RADIO | 33.4783 | 20.6775 | 1.61906** |
| HOUSE | 0.68939 | 14.8145 | 0.04653 |

Number of observation = 161
Log likelihood = -854.3120
Threshold value for the model: Lower = 0.0000 Upper = + infinity
δ = 83.4523
Z = 1.48 ф (z) = 0.2859 Φ(z) = 0.9307

***, **, * indicate significance at 1, 5, and 10% levels, respectively. Source: Model result, 2010.

consumers' surplus if the service is available for free or zero (Figure 2).

## Econometric model result

Econometric software called Limited dependant (Limdep 7) was employed to estimate the Tobit and Probit models. Out of the 16 hypothesized explanatory variables, six were found to be statistically significant, four of them were continuous and the rest two were dummy variables. The variables were age of the household head (AGE), total income from farm (FINC), total off-farm income (OFINC), livestock holding (TLU), owning radio (RADIO), and availability of public and private donations (PAPA). Moreover, the sign of the estimated coefficients were consistent with the expected signs (Tables 4 and 5).

## Conclusion

Age of the household head (AGE) is an important factor

**Table 5.** Marginal effects of the explanatory variable on the dependent variable.

| Variables | Change in probability $\frac{\partial F(z)}{\partial X_i}$ | Change among willing $\frac{\Delta E\left(\frac{y_i}{y_i^*}\right) > 0}{\partial X_i}$ | Total change $\frac{\partial E(y_i)}{\partial X_i}$ |
|---|---|---|---|
| NAWO | -1.66800 | -2.63030 | -2.58250 |
| SEHH | 63.4230 | 100.0140 | 98.1955 |
| AGE | -0.00548 | -1.51590 | -1.4900 |
| EDUC | 0.04564 | 12.6173 | 12.4022 |
| MRST | 13.3687 | 21.0861 | 20.6982 |
| FINC | 0.00002 | 0.0055 | 0.0054 |
| OFINC | -0.00003 | -0.0098 | -0.0096 |
| DEPR | 0.01727 | -4.7743 | -4.6929 |
| FSIZE | 0.01691 | 4.6763 | 4.5966 |
| TLU | -0.01618 | -4.4728 | -4.3966 |
| CREDIT | 0.02439 | -6.7416 | -6.6269 |
| EXTENTION | 0.09502 | 26.2641 | 25.8164 |
| PAPA | -0.16233 | -44.8686 | -46.723 |
| BID | 0.00085 | 0.0235 | 0.0231 |
| RADIO | 0.15218 | 42.0637 | 41.4367 |
| HOUSE | 0.01919 | 5.3063 | 5.2159 |

Source: based on model output.

influences the respondent's willingness to pay negatively, Earlier studies by Patrick (1988), Gine et al. (2007) the age of the household has negative effect on the demand for insurance. Young farmers are more likely to purchase insurance than elders, as the age of household head increases, the willingness to pay amount decreases significantly.

Therefore, younger household heads are more likely to be willing to pay for rainfall risk insurance compared to older household heads. Thus, it might be explained by the fact that younger household heads have less long life experience on predicting weather conditions, and they are also sensitive to the new technologies than elders. The result shows that for each additional year in age of the respondent, the probability of the willingness to pay for rainfall risk insurance decreases by 0.548%. It also shows that as the age of a respondent increase by one year, the amount of cash he/she is willing to pay for rainfall based insurance decreases by 1.5159 Birr.

## Household income from crop (FINC)

This variable is found to have a positive impact on the probability of willingness to pay as hypothesized and the effect is statistically significant at 1% probability level. Those household heads that generate high income from crop production would be more willing to pay for rainfall risk insurance. When the income increases by one birr, the probability of the willingness to pay for the service also increases by 0.002% hence, the income level of the household increase by one Birr. As a result the amount of cash the household could pay increases by 0.0055 Birr, other factors held constant. This is based on economic theory, which states that individual's demand for most commodities or services depend on income (Mbata, 2006). Vince and Joyce (1994) have found that income of the household has positive impact on the demand for rainfall based insurance.

Ownership of radio by the household (RADIO) is another important factor which affect maximum willingness to pay positively. Information from radio enhances the ability of farmers' access to improved technologies and risk management strategies. Farmers that own radio may get different information on extension service, credit service, improved seed variety, input prices and output prices than those farmers who do not have radio. This variable also shows that farmers that own radio have 15.218% of possibility to paying for rainfall risk insurance than those farmers who do not possess. Thus, farmers that own radio would pay Birr 42.0637 more than those farmers that do not have radio.

As expected the availability of off-farm income (OFINC) is negatively related to maximum willingness to pay. Households occupied in different off-farm activities reduce the probability of willingness to pay for rainfall risk insurance by 0.003%. Therefore, this expected to have negative influence on farm activity. A study conducted by Sakurai and Reardon (1997) showed that respondents who received high amount of income from other non-farm activities are not interested in participating in drought insurance. The marginal effect of this variable also shows when off-farm income increases by one Ethiopian birr the amount of money households would be willing to pay for rainfall risk insurance decreases by 0.0098 Birr, other factors held constantly.

### Public and private aid (PAPA)

PAPA is an important factor that affects the dependent variable negatively. Availability of public and private donations decreases the willingness to pay by 16.233%. Sukurai and Reandon (1997) have also found a negative effect on the dependent variable when farmers have found more donations from governmental or other non-governmental organizations, either in kind or cash therefore this might be explained by the fact that as households become more dependent and less active, and their willingness to pay tends to be less. The marginal effect of the variable shows that those household depend on public and private gifts decrease willingness to pay by 44.8686 Birr than those who don't have the gift, other variables held constant.

### Livestock holding (TLU)

Number of livestock ownership is found to have a negative effect. Each additional unit of livestock (TLU) decreases the willingness to pay by 1.618%. The negative effect implies that income from livestock may encourage farmers to depend more on livestock than farming and results in less effort being give to the crop production. The marginal effect shows that for each additional TLU that possess the willingness to pay amount decreases by 4.4728 Birr, other variables held constant.

It was then concluded that if rainfall risk insurance premium is within affordable range (with this range affected by the above mentioned factors) and households have enough information about it, farm households are willing to pay for the service. However, policy makers need to be aware that socio-economic and institution characteristics that are linked with farm households influence the willingness to pay for rainfall risk insurance.

## Conflict of Interest

The authors declared that they have no conflict of interest.

## ACKNOWLEDGEMENTS

The author would like to thank the Almighty and Merciful God, for His help during the course of this study. Also Dr. Ayalneh Bogale is acknowledge for his intellectual stimulation and professional guidance; families and friends for their love, encouragement and financial support. Also not left out are friends at Haramaya University for their valuable support, Mr Ato Fresenbet Zeleke the Head of School of Agricultural Economics and Agribusiness and finally Ato Negeso and all other enumerators for their valuable help during the field survey.

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academicJournals

Vol. 10(28), pp. 2748-2755, 9 July, 2015
DOI: 10.5897/AJAR2015.9848
Article Number: A1E69EB53963
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

*Full Length Research Paper*

# Isolation and characterization of *Bacillus thuringiensis* (Ernst Berliner) strains indigenous to agricultural soils of Mali

**Adounigna Kassogué[1], Kadia Maïga[1], Diakaridia Traoré[1], Amadou Hamadoun Dicko[1], Rokiatou Fané[1], Tiby Guissou[2], Fatouma Alhadji Faradji[1], Fernando Hercos Valicente[3], Abdoulaye Hamadoun[4] and Amadou Hamadoun Babana[1]***

[1]Laboratory of Research in Microbiology and Microbial Biotechnology, Faculty of Science and Technology, University of Sciences, Techniques and Technologies of Bamako, Bamako, BP E320 Mali.
[2]Institut de l'Environnement et de Recherches Agricoles, 03 B.P. 7047 Ouagadougou, Burkina Faso.
[3]Embrapa Milho e Sorgo C.P. 151, CEP: 35701 970 Sete Lagoas, Minas Gerais, Brasil.
[4]Institut d'Economie Rurale (IER), Bamako, Mali.

Received 26 April, 2015; Accepted 3 June, 2015

**The objective of this work was to isolate and characterize *Bacillus thuringiensis* from agricultural and other insect breeding sites in Mali. A hundred soil samples were collected from Bamako district, Segou, Sikasso and Timbuktu regions. *B. thuringiensis* (Bt) was isolated from the samples using a heat-acetate method and the isolates were identified and classified using morphological and biochemical tests. The frequency of *B. thuringiensis* in soils was noted. The results showed that, 15 out of the 3111 bacterial isolates were putative Bt. thuringiensis. Most isolates produced parasporal crystals. The average Bt index for all the areas sampled was 5.1%; the highest frequency was recorded for Niono in Segou region (11.7) and the lowest for Bozola in Bamako district (0.5). Contrary the known information on the high content and distribution of B. in soils, the agricultural soils of Mali contain few Bt strains, confirmed by the low Bt index obtained.**

**Key words:** *Bacillus thuringiensis*, isolation, agricultural soils, Mali.

## INTRODUCTION

In Sub Saharan Africa and especially for Mali, increased rice and maize production are essential to reduce poverty and food insecurity (Colin et al., 2003; Vitale et al., 2007). They are an important staple food, especially for small-holder families with low incomes (Fofana et al., 2008).

However, the productivity of rice and maize plants is threatened by attack from *Orseolia oryzivora* (Harris and Gagné, 1982) and by *Helicoverpa armigera* (Hamadoun et al., 1998; Hamadoun, 1996; Ratnadass and Ajayi, 1995), which has resulted in a drastic reduction in yield from 20 to 40% of the potential yield (Youdeowei, 1989; Seshu-Reddy and Walker, 1990). It is predicted that this decrease in yield will intensify, if no measures are undertaken (FAO, 2013). Presently, the most effective

*Corresponding author. E-mail: ahbabana@laborem-biotech.com.

method of managing these insect pests by small scale farmers is the use of synthetic pesticides (Carneiro et al., 2014). There is the need to develop alternative management strategies because pesticides and are toxic and expensive, and the major pest on maize in Mali, *H. armigera*, have developed resistance to chemical pesticides (Martin et al., 2002). Also, the growing public concern, stricter environmental regulations, and buildup of resistant biotypes to synthetics pesticides have led to an increased interest in alternative environmentally friendly insect management strategies (Vitale et al., 2007).

Several bacterial species act as biological control agents by enhancing plant growth and suppressing insect pest population (Babana et al., 2011; Bathily et al., 2010; Capalbo et al., 2001; Valicente et al., 2008). Among these bacteria, *Bacillus thuringiensis* (Ernst Berliner). *B. thuringiensis (Bt)* a member of a group of crystalliforeus spore forming aerobic, Gram-positive bacteria (Larison, 2006), is found worldwide in a variety of habitats including soil (Carozzi et al., 1991), insects (Carozzi et al., 1991, stored-product dust (Burges and Hurst, 1977), and some plant leaves (Kaelin et al., 1994). Very little is known about the natural ecology of Bt other than that it occurs naturally in many soils. One study of Entwistle (1993) indicated a little capacity of Bt to move laterally in soil. Other studies of Entwistle (1993) found that Bt was not recovered past a depth of 6 cm after irrigation.

The insecticidal activity of Bt was initially discovered in 1911. Some Bt strains produces parasporal crystals containing one or more Cry proteins that may be toxic for different insect orders including the ones damaging agricultural plants and products (Maruthesh, 2007), but its has only been developed on commercial scales over the last 40 years (Capalbo et al., 2001). Yang and Wang (1998) showed that pest control programs management using Bt pesticides result in a reduction of the use of chemical insecticides. Successful applications have been documented in a variety of agriculturally important crops such as cotton, corn, potato, soybean and many vegetables by Yang and Wang (1998). Bt biopesticides could be an alternative to synthetic insecticides that often have unintended harmful effects on non-target insect species. In spite of the importance of *B. thuringiensis* for the management of major agricultural insect pest, and its high potential for the management of *H. armigera* Hübner in maize and *Orselia orizyvora* in rice, there are little information on the quantity and diversity of *B. thuringiensis* in Malian agricultural soils has not been explored in Mali. That why the authors proposed in collaboration with EMBRAPA a proposal funded by Marketplace Africa-Brazil to develop a biopesticide. There is therefore the need to explore the presence diversity and activity indigenous of Bt and to use the efficient method developed by EMBRAPA (Capalbo et al., 2001; Valicente et al., 2008) to formulate biopesticide based on *B. thuringiensis* (Bt) toxins for the biological control maize and rice pests. Hence, this study aimed at isolating and characterizating *B. thuringiensis* from agricultural and other insect breeding sites in Mali. These could potentially be formulated into biopesticides for the management of insect pest of maize and rice.

## MATERIALS AND METHODS

### Soil sample collection

The samples were collected by scraping off surface material with a sterile spatula and then obtaining a 10 g sample 2 to 5 cm below the surface. Soil samples were taken from locations as diverse as ditches, wetlands and soils under maize, rice, cotton and bean. All samples were placed in sterile plastic bags aseptically and stored at 4°C until processed.

### Isolation and characterization of *B. thuringiensis* strains

#### *Isolation of B. thuringiensis strains*

Isolation of *B. thuringiensis* strains was conducted according to the method described by Travers et al. (1987). One gram of each sample was suspended in 10 ml sterile distilled water and pasteurized at 80°C for 30 min. For the selection of *B. thuringiensis,* 1 ml of each suspension was added to 10 ml of Luria-Bertani (Merck, Germany) and broth buffered with 0.25 M sodium acetate at a pH of 6.8. The suspensions were incubated at 30°C for 4 h and then heated at 80°C for 4 min. Suspensions were diluted and plated on T3 medium containing per liter: 3 g tryptone*, 2 g* tryptose, 1.5 g yeast extract, 0.05 M sodium phosphate of pH 6.8, and 0.005 g of $MnCl_2$. After incubation at 30°C for 24 h, the colonies showing similar morphology were selected and examined under phase-contrast microscope to determine the presence of parasporal inclusions and spores. The reference strains *B. thuringiensis* HD-125 (UNAM, Mexico) and *B. thuringiensis* T09 (Institut Pasteur-France), were supplied by Dr. Fernando Hercos Valicente (Embrapa Milho e sorgho, Brazil).

#### *Characterization of B. thuringiensis strains*

Bacterial isolates was characterized according to Laridon (2006).

**Colony morphology:** The colonies which were found to be white to off-white in colour with smooth edges and flat to slightly raised elevation (Rampersad and Ammons, 2002) were selected and marked on the Petri dish. The selected colonies with those characteristics were categorized as possible Bacillus colonies. Total of 144 colonies were chosen based on colony morphology. These selected colonies were then sub-cultured onto new Nutrient Agar plates and incubated.

**The gram stain test:** A very small inoculum of bacteria was smeared onto a clean slide using an inoculation loop. The sample was diluted with a drop of sterile distillated water and allowed to air dry. It was then heat-fixed by passing the slide through an open flame. The slide was stained with crystal violet for 1 min and rinsed with sterile water. The slides were then stained with Gram's iodine (1% iodine, 2% potassium iodide in water) for 1 min to fix the dye and then rinsed with sterile water. Excess stain was removed with 95% ethanol and then rinsed with sterile water. Specimens were counterstained with Safranin for 1 min, rinsed with water and then air dried. Slides were viewed using light microscopy under oil immersion (Provine and Gardner, 1974; Bergey's Manual of Systematic Bacteriology, 1986).

**Endospore stain (Schaeffer-Fulton staining method):** A small inoculum of bacteria was smeared onto a clean slide using an inoculation loop and diluted with a drop of sterile water. Once it was dry the slides were flooded with Malachite green (made by dissolving 5.0 g in distilled water, made up to 100 ml) and immediately steamed over a water bath for 5 min. After cooling, the slides were rinsed with sterile water. The slides were then counterstained with Safranin O (made by dissolving 0.5 g Safranin O powder in distilled water, made up to 100 ml) for 2 min and then rinsed with sterile water. The specimens were viewed under a compound microscope with oil immersion after the slides had dried (Mormak and Casida, 1985; Bergey's Manual of Systematic Bacteriology, 1986).

**Catalase test:** The test involved adding hydrogen peroxide to each sample of bacteria. 33 µl of 48 h Bt cultures was smeared onto a clean slide and a drop of 10% hydrogen peroxide solution was alloquoted onto the Bt smear and observed using light microscopy. A slide smeared with inoculum free LB media was used as the negative control, and an inoculum of *B. thuringiensis* HD125 was used as the positive control. The slides were analyzed for the formation of oxygen bubbles and photographed using a digital camera. The presence of bubbles indicated the ability to break down hydrogen peroxide into water and oxygen (Bergey's Manual of Systematic Bacteriology, 1986).

**Growth above 45°C:** All samples were diluted in order to obtain an optical density (OD) reading of 0.3. Spectrophotometer readings were taken with an absorbance of 600 nm (A600) for each sample prior to incubation, and then once daily for a period of 5 days to determine if growth occurred. Isolated samples were incubated in nutrient broth at a temperature exceeding 45°C for a 5 day period. Cultures that showed signs of growth and thus were capable of reproducing at such high temperatures were assumed not to be *B. thuringiensis* and were eliminated as putative Bt isolates (Laridon, 2006).

**Presence of parasporal bodies (δ-endotoxins):** The presence of parasporal bodies was confirmed using phase-contrast microscopy. Vegetative cells and parasporal bodies were observed on slides freshly coated with a thin layer of 2% water agar (200 mg biological grade agar diluted in 100 ml sterile water). A drop of culture was placed on the slide and observed using phase-contrast microscopy under a 100X oil immersion objective. Parasporal bodies were characterized as either bipyramidal, spherical, rectangular (cuboid), irregular spherical, or irregularly pointed.

## RESULTS AND DISCUSSION

### *B. thuringiensis* isolates

The results of the analysis of the 100 samples are shown in Table 1. Observations under a light microscope showed a total of 3111 Bacillus-like colonies out of which 38 isolates (4.7%) were identified as putative *B. thuringiensis* (Bt). These isolates were observed to show the staining proteinaceous crystals characteristic of Bt species. According to the definition of the Bt index which is considered to be the number of identified Bt colonies divided by the total number of Bacillus like colonies examined, the authors obtained an average Bt index of 1,22 for agricultural soils in Mali (Table 1). The Bt index in Malian agricultural soils is very low as *B. thuringiensis* is qualified as an ubiquitous bacterium, found most abundantly in soil habitats all around the world (Theunis et al., 1998; Martin and Travers, 1989). ICRISAT and Niono sampling sites showed the highest Bt index with 7.4 and 11.76, respectively, followed by Fanidiama, Bougouni, Cinzana, CAA, Samanko et Daoudabougou with 2.9, 2.8, 2.7, 2.5, 2.2, 2.1; respectively. Bozala and Dire sampling sites showed the lowest Bt index with 0.5 and 0.7, respectively (Table 1). These results are not in accordance with that of Bernhard et al. (1997), who found Bt isolates abundantly in plant storage systems, mushrooms, soils, compost as well as deciduous and coniferous leaves

### Colony morphology

A total of 3111 Bacillus-like were isolated. Colonies with white to off-white color, smooth edges and flat to slightly raised elevation were isolated and sub cultured in fresh Nutrient Agar plates to obtain single colonies (Figure 1). Thirty-eight colonies with those characteristics was categorized as possible *Bacillus* colonies. In 100 soil samples, we obtained only 38 Bt isolates. Contrary to this result, Laridon (2006), working on 30 soil samples collected in tundra plant comminuties recovered 127 *B. thuringiensis* after the examination of 238 bacterial colonies recovered.

### Gram staining

*Bacillus* species are generally gram positive and rod-shaped (Figure 1). Gram staining was done to differentiate gram positive from the gram negative. Light microscope was used for observations. Isolates which were rod shaped and blue in color indicates a gram positive strain whereas isolates which did not exhibit these characteristics were discarded.

Under the microscope, the vegetative cells of *Bacillus* are thin and long. All samples isolated from morphological characterization and sodium acetate selections tested as Gram positive (Table 2) rod shaped bacteria (Figure 2).

### Phase contrast microscopy

Phase contrast is carried out after determining the colonies are gram positive through gram staining. This procedure is important to confirm the isolates are *Bacillus* by viewing the endospore and parasporal bodies. Besides that, phase contrast microscopy was also done for vegetative phase cells to confirm the isolates were rod shape (Figure 2). The authors mounted slides prepared

**Table 1.** Isolation of *Bacillus thuringiensis* from Malian soil samples.

| Sampling site | Bacillus-like isolates | Total Bt isolates | Bt index |
|---|---|---|---|
| Bozola (B) | 2181 | 12 | 0.5 |
| Daoudabougou (D) | 375 | 8 | 2.1 |
| Samanko (S) | 46 | 1 | 2.2 |
| CAA (C) | 80 | 2 | 2.5 |
| ICRISAT (I) | 54 | 4 | 7.4 |
| Cinzana (C) | 73 | 2 | 2.7 |
| Niono (N) | 34 | 4 | 11.76 |
| Dire (Di) | 130 | 1 | 0.7 |
| Fanidiama (F) | 68 | 2 | 2.9 |
| Bougouni (Bo) | 70 | 2 | 2.8 |
| Total | 3111 | 38 | 1.22 |

**Table 2.** 15 bacteria samples isolated from agricultural were identified as putative *Bacillus thuringiensis* isolates.

| Sample name | Source | Mobility | Gram stain | Spore stain | Catalase | Growth over 45°C | Parasporal bodies |
|---|---|---|---|---|---|---|---|
| $D_3G$ | Soil | + | + | + | + | - | + |
| $B_9G$ | Soil | + | + | + | + | - | + |
| $CBt_1$ | Soil | + | + | + | + | - | + |
| $IBt_1$ | Soil | + | + | + | + | - | + |
| $DBt_2$ | Soil | + | + | + | + | - | + |
| $SBt_1$ | Soil | + | + | + | + | - | + |
| $BBt_1$ | Soil | + | + | + | + | - | + |
| $NBt_1$ | Soil | + | + | + | + | - | + |
| $NBt_3$ | Soil | + | + | + | + | - | + |
| $D_1G$ | Soil | + | + | + | + | - | + |
| $B_1P$ | Soil | + | + | + | + | - | + |
| B1G | Soil | + | + | + | + | - | + |
| B9P | Soil | + | + | + | + | - | + |
| D4 | Soil | + | + | + | + | - | + |
| B5 | Soil | + | + | + | + | - | + |

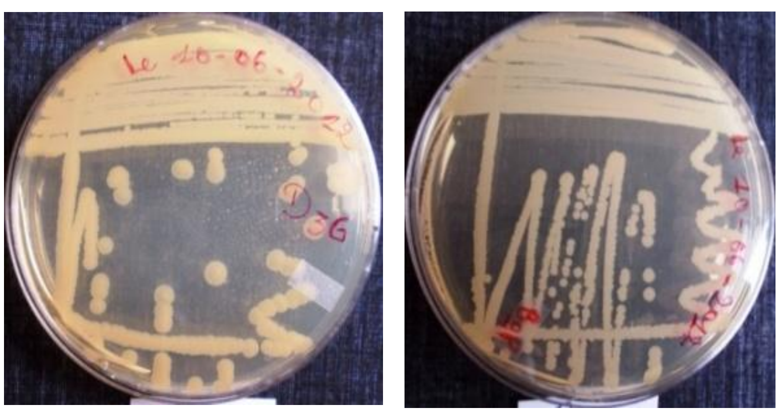

**Figure 1.** Single creamy and white colonies obtained after sub-culturing of *Bacillus thuringiensis* D3G (right) and B9P (right).

after 4 days of incubation for each isolate. Isolates that showed presence of both endospores and parasporal bodies were selected for further characterization and isolates without those characteristics were discarded (Figure 3). A total of 15 isolates possessed endospores and parasporal bodies.

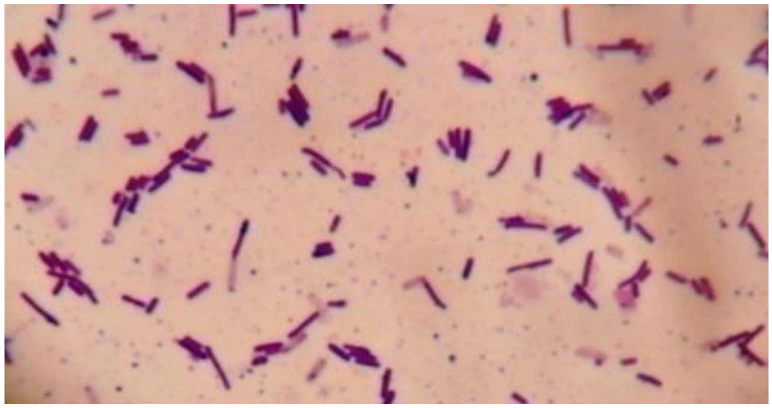

**Figure 2.** Vegetative form of *Bacillus* (rod shaped and thin) at 1000× magnification. Gram positive (blue).

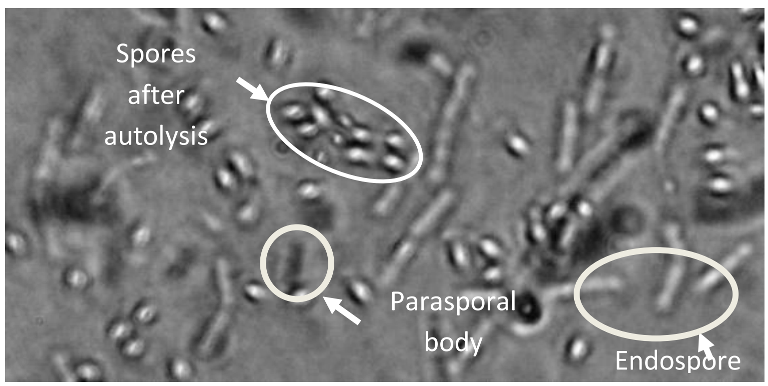


**Figure 3.** Sporulated form of Bacillus at 1000× magnification. Parasporal body is a dark oval region and endospores are bright rod shape.

Total of 15 isolates were subjected to further screening of parasporal body through coomassie blue staining (Figure 5). This method has a higher resolution compared to phase contrast microscopy. Thus, samples which have parasporal body can be easily identified. All 15 isolates took up the coomassie blue stain (Figure 4) and had parasporal bodies during the sporulated phase and autolysis phase (Table 2).

## Growth above 45°C

After 24 h of incubation, 61% of the 39 tested bacteria showed evidence of growth. Growth was not observed in the remaining samples after 24 h; hence, these were excluded from further tests.

## Catalase test

The catalase test was performed on all isolates that tested positive for selection tests including the morphological characterization and the sodium acetate selection test (Table 2). All of the samples tested were positive for catalase degradation. After the introduction of hydrogen peroxide to the bacterial smears, samples underwent a violent reaction with bubbles forming at rapid rates (Figure 6). The negative control showed no activity.

In our experiments, we used the sodium acetate test, which proved to be more successful in eliminating most non-wanted bacteria (most sporeforming and nonsporeforming organisms) in the test soil samples. Nevertheless, as observed by Travers et al. (1987), all of the isolates tested positive for Gram stain, endospore stain and catalase test, confirming the necessity to use heat shock to eliminate nosporforming and vegetative cells protected by endospores.

This study used traditional *Bacilli* identification approaches to assign isolates into different groups: aerobic, gram positive, rod shaped bacteria with endospore formation. The different bacteria were further

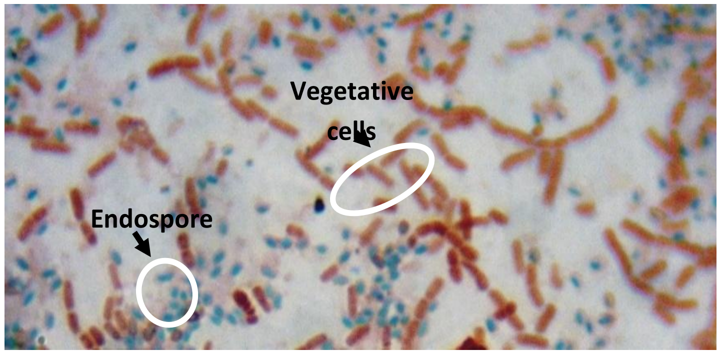


**Figure 4.** Digital photographs of isolates with green endospores stained with malachite green are distinguishable from the pink safranin O stained vegetative tissue of living bacterial cells (taken at 100x magnification with oil immersion).

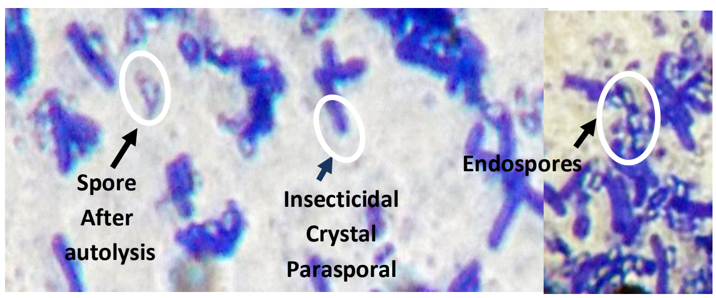


**Figure 5.** Sporulation phase of Bt. isolates with blue stained parasporal body at 1000X magnification.

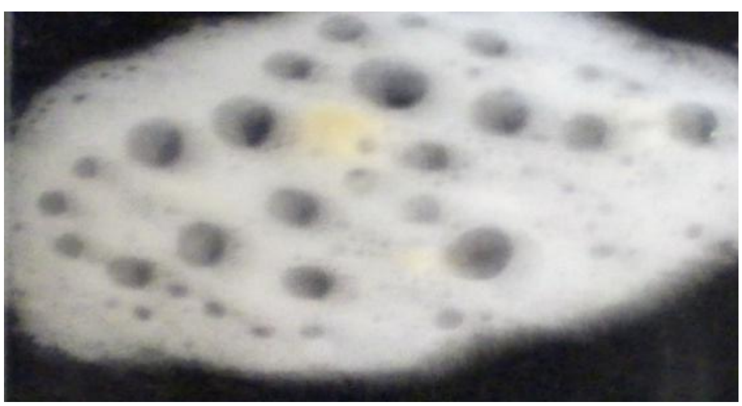

**Figure 6.** Bubble production from bacterial smear in the presence of Hydrogen peroxide.

identified to the species level using physiological and biochemical tests. Also, familiarity with these bacteria was necessary in order to distinguish spore morphologies (Claus and Berkley, 1986). The total bacteria isolated from agricultural soils of Mali were 3111 out of which only 15 were considered as putative *B. thuringiensis*. These results suggest that although many bacteria were isolated from these soils, only few strains could be considered as putative *B. thuringiensis.* Similar results were reported by Deacon (2001) and Zhang et al. (2000) when they isolated many organisms from the natural habitats of insect pests but found only few *B. thuringiensis*.

Also, our result showed Bt indexes were between 0.5 (in Bozola) and 11.7 (in Niono) with an average of 5.1. The higher Bt index in Niono can be explained by the fact that Niono is the highest rice and vegetable production area in Mali. In a *B. thuringiensis* isolation and identification study, Keshavarzi (2008) reported Bt indices between 0 and 5.1 with an average Bt index of 3.2, which was below that reported in the present study.

## Conclusion

This work shows that that many bacteria exist in agricultural soils of Mali, but only few strains could be considered as putative *B. thuringiensis.* The Bt index is highly variable in agricultural soils in Mali, but agricultural soils from Niono, the important rice and vegetable production zone in Mali, show the highest Bt index.

## Conflict of Interest

The authors have not declared any conflict of interest.

## ACKNOWLEDGEMENTS

Adounigna Kassogue is a doctoral student at the University of Sciences, Techniques and Technologies of Bamako. His research work is supported by a Grant by Marketplace Africa-Brazil as a project entitled ″Enhancing small-holder rice and maize production using bacteria-plant extract biopesticide.″

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Vol. 10(28), pp. 2756-2762, 9 July, 2015
DOI: 10.5897/AJAR2015.9778
Article Number: 49B51FD53957
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

*Full Length Research Paper*

# Modelling crown volume in *Acacia mearnsii* stands

**Carlos Roberto Sanquetta[1]*, Alexandre Behling[1], Ana Paula Dalla Corte[1], Augusto Arlindo Simon[2], Aurélio Lourenço Rodrigues[1], Guilherme Camacho Cadori[1] and Sérgio Costa Junior[1]**

[1]Universidade Federal do Paraná, Laboratório de Inventário Florestal (LIF), Centro de Excelência em Pesquisas sobre Fixação Carbono na Biomassa (BIOFIX), Av. Pref. Lothário Meissner, 900 - Jardim Botânico - Campus III, CEP: 80210-170 - Curitiba - Paraná – Brasil.
[2]TANAC S.A. Departamento Florestal, Rua Torbjorn Weibull, 199 – Tanac, CEP: 95780-000, Montenegro – Rio Grande do Sul – Brasil.

Received 31 March, 2015; Accepted 20 May, 2015

**The aim of the present study was to model the crown volume in black wattle stands (*Acacia mearnsii* De Wild.) in Rio Grande do Sul, Brazil. The study was carried out in plots installed in black wattle stands in area where the plantations of the species are common. Two trees in each plot of average diameter were felled to determination total height, crown length (crown height) and crown width taken twice, each measurement oriented perpendicular to the other across the bole axis and parallel to the base of the crown. To calculate crown volume (cv) widths were taken every meter starting at the base of the tree bole. Stepwise, backward, and forward variable selection methods were used to formulate the volume equations. The combined variable squared diameter and crown height ($d^2ch$) was the most highly correlated with crown volume (r=0.84). This in turn was the variable that was integrated into the selected model, both in the stepwise and forward selection methods. The equation cv = $b_1$ $d^2ch + \varepsilon i$ was the model that provided the best fit for predicting crown volume in black wattle stands, both in age rotation and in young stands.**

**Key words:** *Acacia mearnsii;* morphometry; stepwise*;* models.

## INTRODUCTION

Black wattle (*Acacia mearnsii* De Wild.) is a tree species which is prominent today in the state of Rio Grande do Sul, and stands of the species rank among the most widely planted in region behind the genera *Eucalyptus* and *Pinus*. According to Simon (2005) black wattle is the primary source of bark for the global plant-based tannin industry, used mostly in leather tanning. The high-quality wood from this species is ideal for pulp and paper production, and most of the wood is consumed in these industries (Stein and Tonietto, 1997). The species is cultivated by more than 10,000 small farms and therefore plays an important socioeconomic role in the region (Oliveira et al., 2006).

One of the characteristics of black wattle stands is the use of intercropping, especially in the early years including annual crops, such as watermelon, corn and cassava, and in later years along with cattle (Fleig et al., 1993; Mora, 2002; Muller, 2006). In intercropping systems where the soil nutrient conditions, temperature and water availability are not limiting factors and pests

*Corresponding author. E-mail: carlos_sanquetta@hotmail.com

and disease are not significant factors, growth and yield of intercropped species depends primarily on the total solar radiation intercepted by the plants during the growing period (Monteith 1978). Therefore, knowing the amount of light that reaches the plant system is critical for managing intercropped species (Sinoquet and Bonhomme, 1992).

The amount of radiation intercepted in a stand is determined by the space that the crown occupies, which can be determined by its volume. Once determined, the crown volume can be used for various purposes, such as predicting the amount of light reaching the interior of the stand (important in the case of intercropping) or the amount of light intercepted by the crown (a factor that regulates growth and production of the population), and also as an input variable in simulating growth and crop yield (Meng et al., 2007).

The crown profile and volume are generally obtained by making approximations based on geometric figures such as cones, whose volumes are easily calculated. However, models using simple geometric shapes do not accurately represent the actual volume of the crown, since in most cases the crown departs from commons geometric shapes (Durlo et al., 2004; Crecente-Field, 2008). An alternative to this procedure is to estimate the volume of the tree crown and fit models with independent variables whose data are easily obtained, and so making the process less costly and more reliable.

However, the great difficulty in fitting a model well lies in meeting the assumptions required of a regression, such as parameter linearity, normality, homoscedasticity and the absence of error autocorrelation. Furthermore, mean errors of the regression model should be zero and the regressed variables should not exhibit collinearity (required exclusively of multiple regressions), according to Werkema and Aguiar (2006) and Hair Jr. et al. (2009). Such requirements in the regression analysis have not been well researched, though it is an essential procedure for assessing the quality of fit and the reliability of the model.

In light of the importance of estimating crown volume and finding models that meet the statistical requirements, this study sought to model crown volume in black wattle stands.

## MATERIALS AND METHODS

To conduct this study we drew data from temporary plots installed in commercial plantations of black wattle in regions of high concentrations of the species in the state of Rio Grande do Sul, Brazil, in the municipalities of Cristal and Piratini. In each region, stands were studied at the end of the rotation, or at seven years old.

In each stand a North-facing slope was selected where one plot was demarcated in the each of the upper, middle and bottom thirds of the slope. The plot size was 9 m wide by 14 m long, with four rows of 10 plants per row.

In each plot the circumference at breast height was measured for all trees using a graduated tape measure. Two trees of average diameter (d) in each plot were selected for morphometric variable evaluation. This procedure was adopted to gather additional data for use in other studies that made use of this data set.

The morphometric assessment consisted of measuring (using a tape measure) the total height (H), length of the crown (plant height) and crown width twice measured, each measurement oriented perpendicular to the other across the bole axis and parallel to the base of the crown.

The calculation of crown volume was performed using the following expression:

$$cv = \left(\frac{a_1 + a_2}{2}\right) l + \left(\frac{a_2 + a_3}{2}\right) l + \dots + \left(\frac{a_{n-1} + a_{sn}}{2}\right) l$$

Where: cv = sum of the volumes through to the last section of the crown ($m^3$); $a_1$ = crown area section 1 ($m^2$); $a_2$ = crown area section 2 ($m^2$); $a_3$ = crown area section 3 ($m^2$); $a_{n-1}$ = crown area of the penultimate section ($m^2$); $a_n$ = crown area of the last section ($m^2$); and l = length of section (m). To calculate the cross-sectional area of the crown in each section (a) we used the following expression:

$$a = \frac{\pi * dc^2}{40000}$$

Where: a = cross-sectional area of the crown ($m^2$) and dc = diameter of the crown of the corresponding section (m).

The last measured section was considered a cone, and the formula for the volume of a cone was used to make the calculation, where:

$$v_{co} = \frac{1}{3} * a * lc$$

Where: $v_{co}$ = the volume of the cone of the crown ($m^3$); a = cross-sectional area of the last section "n" ($m^2$), and lc = length of the cone. Thus, the total volume of the crown of each individual was calculated by summing the volume of all sections plus the volume of the cone.

We used stepwise, backward and forward variable selection methods (Draper and Smith, 1966) to obtain equations to estimate crown volume at 5% error probability. The three methods were tested while bearing in mind that the result of the variable selection may not always be the same. Statistical analyses were conducted in SAS 8.1 (SAS, 2002).

The independent input variables in the models were: diameter at breast height (d, in cm), total height (h, in meters) and crown height (ch, in meters), as well as combinations of these variables and log transformations. The dependent variable in the studied models was the crown volume (vc, in cubic meters).

Initially, a Pearson correlation analysis was performed between crown volume and dendrometric variables, both in their original, combined and transformed form. The results of formed the basis for the use of stepwise, forward, and backward selection methods. The *t* test, at a 5% probability level, was used to verify the existence of a linear relationship between variables.

The equations were evaluated and selected by: adjusted coefficient of determination ($R^2_{adj}$, in %), the standard error of estimate ($S_{yx}$, in %), F value for $p < 0.05$, and the significance of all the coefficients and the residual plot as a function of the estimated values (%).

The models generated were also tested with respect to the regression conditions using the White test (homoscedasticity), Shapiro-Wilk test (normality) and Durbin-Watson test (independence). For the multiple linear models, a tolerance level was determined and consequently a variance inflation factor (VIF), since the latter is an indicator of the effect that other independent variables have on the standard error of a regression coefficient. High VIF values (greater than 10) indicate a high degree of collinearity or multicollinearity (Hair Jr. et al., 2009), or increasing variance in the coefficients given by the correlation between the independent variables.

The performance of the best model, using the procedures described above, was tested in varying age of black wattle stands. Therefore, further evaluations were conducted in the same manner and used in the construction of the model, in one, three and five year old stands. The evaluation of fits was based on the cited accuracy statistics as well as the chi-square test ($\chi^2$) at 5% probability.

Therefore, models were constructed using 12 trees from 6 plots, and the performance was evaluated based on 36 other trees from another 18 plots.

## RESULTS AND DISCUSSION

Using simple linear correlation analysis, squared diameter and crown height ($d^2ch$) combined variable was found to be the most highly correlated with crown volume (r = 0.84). The other variable significantly correlated with crown volume was diameter at breast height (d), both untransformed and transformed as 1/d, ln d, and ln 1/d (r = 0.80, -0.80, 0.80 and -0.80, respectively). Other variables showing significant correlations with crown volume were the combination of the squared diameter with the total height ($d^2ht$) and crown height (ch).

The existence of a high correlation between the tree crown diameter with bole diameter is already widely understood (Hemery et al., 2005) and based on the results obtained it is clear that the diameter of the bole also has a significant correlation with the crown volume. Meng *et al*. (2007) modelled the crown volume of *Pinus contorta* Engelm. and found that the diameter at breast height had the strongest independent correlation with crown volume, accounting alone for 62% of the total variation. But the fact that the variable $d^2ch$ presented the highest correlation with the volume is justified since it generated a virtual parallelogram with respect to the squared diameter, referring to the area occupied by the crown. The variable is combined with the height of the crown, referring to its length and therefore, strongly correlated with volumetric form.

The transformation of all the independent variables did not improve the correlation with crown volume. Therefore, with the aim of compiling a single matrix mainly for the backward method, these variables were excluded from the process. Thus, total height (h), crown height (ch), diameter at breast height (d) and the combination between the diameter (d) squared with the total height ($d^2ht$) and diameter (d) squared with crown height ($d^2ch$) were all part of the modelling process.

With the inclusion of the independent variable most highly correlated with crown volume ($d^2ch$), the stepwise variable selection procedure generated a single step, resulting in the equation cv = - 0.56767 + 0.01467 $d^2ch$ (Table 1).

It was observed that the coefficient $b_o$ of the obtained equation was not significant (p> 0.05), resulting in an increase in the standard error of the coefficients. Thus the exclusion of this coefficient was tested, resulting in the equation cv = 0.01431 $d^2ch$. The change yielded an improvement of approximately 11% in the adjusted coefficient of determination ($R^2_{adj}$), lower standard error of the estimate ($S_{yx}$), and reduced standard error (and consequently an improved confidence interval) of the coefficient.

Comparing the two equations, both showed adequate distribution of residuals (Figure 1a), and met the conditions of the regression tested, given the non-significance (p> 0.05) of the White, Shapiro-Wilk and Durbin-Watson tests. Since the improvement of statistical fit was obtained in the model without intercept, the following model was obtained by the stepwise method: cv = $b_1 d^2ch + \varepsilon i$

The backward variable selection method resulted in two steps: the first with the full model (including all variables) and the second where the crown height variable was excluded. The statistics obtained for the two steps in the fit are shown in Table 1. The fits resulted in a difference of less than 1% with respect to $R^2_{adj}$ and $S_{yx}$. Both equations also showed adequate distribution of residuals along the estimated line and a similar trend (Figure 1).

In the first step, the coefficients $b_3$ and $b_5$ were not significant and were excluded from the model. However, when the ch variable was excluded in the second step, all coefficients of the model become significant (p <0.05) resulting in the equation:

$$cv = -572.58561 + 14.71806\, h + 43.69779\, d - 0.08388\, d^2h + 0.01103\, d^2ch.$$

The results of the White, Shapiro-Wilk, and the Durbin-Watson tests for the model obtained by the backward method (cv = $b_0 + b_1 h + b_2 d + b3\, d^2h + b_4 d^2ch + \varepsilon i$) were not significant, indicating that the model met the requirements of normality, homogeneity of variances and independence.

The values of the variance inflation factor (VIF) and the high linear correlations found between the independent variables present in two steps suggest a multicollinearity effect in the multiple regression proposed by this method, because the VIF values are greater than 10 (Table 1). Multicollinearity occurs when any independent variable is highly correlated with a number of other independent variables present on the same model as in the present case is between DBH, ch, and the combination $d^2ch$.

The forward variable selection method resulted in three steps: The first began with the independent variable most highly correlated with volume and therefore the same equation obtained by the stepwise method. In the next two steps the variables diameter at breast height and crown height were added (Table 1).

The three steps resulted in similar fits, with $R^2_{adj}$

**Table 1.** Statistical accuracy of models obtained by stepwise, forward, and backward methods to estimate crown volume in black wattle.

| Model | | | $R^2_{adj}$ | | $S_{yx}$ | F | α (F) | W | SW | DW |
|---|---|---|---|---|---|---|---|---|---|---|
| | $cv = b_0 + b_1 d^2ch + \varepsilon i$ | | 67.86% | | 17.37% | 24.23 | 6.03E-04 | $3.08^{ns}$ | $0.97^{ns}$ | $1.91^{ns}$ |
| | $cv = b_1 d^2ch + \varepsilon i$ | | 88.58% | | 16.58% | 463.00 | 1.05E-09 | $2.8^{ns}$ | $0.97^{ns}$ | $1.95^{ns}$ |
| | | Coefficients | Syx | t | α (t) | | | Confidence Interval | | |
| Stepwise method | $cv = b_0 + b_1 d^2ch + \varepsilon i$ | | | | | | | | | |
| | $b_0$ | -0.56767 | 4.63 | -0.122 | 0.9 | -10.90 | | ≤ Y≤ | 9.76 | |
| | $b_1$ | 0.01467 | 0.00 | 4.922 | 6.4E-04 | 0.00 | | ≤ Y≤ | 0.02 | |
| | $cv = b_1 d^2ch + \varepsilon i$ | | | | | | | | | |
| | $b_1$ | 0.01431 | 6.65E-04 | 21.51 | 2.4E-10 | 0.01 | | ≤ Y ≤ | 0.01 | |
| | | Model | $R^2_{adj}$ | $S_{yx}$ | F | α (F) | | W | SW | DW |
| | $cv = b_0 + b_1 h + b_2 d + b_3 ch + b_4 d^2h + b_5 d^2ch + \varepsilon i$ | | 88.00% | 10.62% | 17.140 | 1.70E-03 | | $12.00^{ns}$ | $0.96^{ns}$ | $1.98^{ns}$ |
| | $cv = b_0 + b_1 h + b_2 d + b_3 + d^2h + b_4 d^2ch + \varepsilon i$ | | 87.16% | 10.99% | 19.660 | 6.58 E-04 | | $12.00^{ns}$ | $0.88^{ns}$ | $1.74^{ns}$ |
| | | Coefficients | $S_{yx}$ | t | α (t) | Confidence Interval | | | T | VIF |
| | $cv = b_0 + b_1 h + b_2 d + b_3 ch + b_4 d^2h + b_5 d^2ch + \varepsilon i$ | | | | | | | | | |
| | $b_0$ | -530.8521 | 151.29 | -3.50 | 1.2E-02 | -901.04 | ≤ Y ≤ | -160.65 | - | 0 |
| | $b_1$ | 19.5751 | 5.66 | 3.45 | 1.3E-02 | 5.72 | ≤ Y ≤ | 33.42 | 0.004 | 233 |
| | $b_2$ | 40.5999 | 11.32 | 3.58 | 1.2E-02 | 12.90 | ≤ Y ≤ | 68.29 | 0.008 | 125 |
| Backward method | $b_3$ | -12.5966 | 10.30 | -1.22 | 2.7E-01 | -37.80 | ≤ Y ≤ | 12.60 | 0.001 | 643 |
| | $b_4$ | -0.1166 | 0.03 | -3.34 | 1.6E-02 | -0.20 | ≤ Y ≤ | -0.03 | 0.001 | 600 |
| | $b_5$ | 0.0922 | 0.06 | 1.38 | 2.2E-01 | -0.07 | ≤ Y ≤ | 0.25 | 0.000 | 1341 |
| | $cv = b_0 + b_1 h + b_2 d + b_3 d^2h + b_4 d^2ch + \varepsilon i$ | | | | | | | | | |
| | $b_0$ | -572.5856 | 152.51 | -3.75 | 7.1E-03 | -933.23 | ≤ Y ≤ | -211.94 | - | 0 |
| | $b_1$ | 14.7180 | 4.17 | 3.52 | 9.6E-03 | 4.85 | ≤ Y ≤ | 24.58 | 0.008 | 118 |
| | $b_2$ | 43.6977 | 11.41 | 3.82 | 6.5E-03 | 16.70 | ≤ Y ≤ | 70.69 | 0.008 | 118 |
| | $b_3$ | -0.0838 | 0.02 | -3.63 | 8.4E-03 | -0.13 | ≤ Y ≤ | -0.02 | 0.004 | 244 |
| | $b_4$ | 0.0110 | 0.00 | 2.92 | 2.2E-02 | 0.00 | ≤ Y ≤ | 0.01 | 0.249 | 4 |
| | | Model | $R^2_{adj}$ | Syx | F | α (F) | | W | SW | DW |
| | $cv = b_0 + b_1 d^2ch + \varepsilon i$ | | 67.86% | 17.38% | 24.230 | 6.03E-04 | | $3.08^{ns}$ | $0.97^{ns}$ | $1.91^{ns}$ |
| | $cv = b_0 + b_1 d + b_2 d^2ch + \varepsilon i$ | | 70.44% | 16.67% | 14.107 | 1.68E-03 | | $4.73^{ns}$ | $0.95^{ns}$ | $2.14^{ns}$ |
| | $cv = b_0 + b_1 d + b_2 ch + b_3 d^2ch + \varepsilon i$ | | 72.38% | 16.11% | 10.607 | 3.67E-03 | | $5.55^{ns}$ | $0.90^{ns}$ | 1.52* |
| | | Coefficients | $S_{yx}$ | t | α (t) | Confidence Interval | | | T | VIF |
| Forward method | $cv = b_0 + b_1 d^2ch + \varepsilon i$ | | | | | | | | | |
| | $b_0$ | -0.56767 | 4.63 | -0.12 | 9.1E-01 | -10.90 | ≤ Y ≤ | 9.76 | - | - |
| | $b_1$ | 0.01467 | 0.00 | 4.92 | 6.1E-04 | 0.00 | ≤ Y ≤ | 0.02 | - | - |
| | $cv = b_0 + b_1 d + b_2 d^2ch + \varepsilon i$ | | | | | | | | | |
| | $b_0$ | -38.17384 | 27.84 | -1.37 | 2.0E-01 | -101.06 | ≤ Y ≤ | 24.81 | - | 0 |

**Table 1.** Contd.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| $b_1$ | 3.42212 | 2.50 | 1.36 | 2.0E-01 | -2.23 | ≤ Y ≤ | 9.08 | 0.47 | 2 |
| $b_2$ | 0.00988 | 0.00 | 2.19 | 5.6E-02 | 0.00 | ≤ Y ≤ | 0.02 | 0.47 | 2 |
| | | | $cv = b_0 + b_1 d + b_2 ch + b_3 d^2ch + \varepsilon i$ | | | | | | |
| $b_0$ | -183.76743 | 117.14 | -1.56 | 1.6E-01 | -453.91 | ≤ Y ≤ | 86.37 | - | 0 |
| $b_1$ | 14.84143 | 9.26 | 1.60 | 1.5E-01 | -6.52 | ≤ Y ≤ | 36.20 | 0.02 | 37 |
| $b_2$ | 8.39067 | 6.57 | 1.27 | 2.4E-01 | -6.76 | ≤ Y ≤ | 23.54 | 0.00 | 115 |
| $b_3$ | -0.04108 | 0.04 | -1.02 | 3.4E-01 | -0.13 | ≤ Y ≤ | 0.05 | 0.00 | 214 |

Where: W = White, SW = Shapiro-Wilk, DW = Durbin-Watson, T = tolerance and VIF = variance inflation factor.

variation of less than 5% and $S_{yx}$ variation of less than 1.5% and a similar trend in the distribution of the residuals (Figure 1). Despite the significant values of *F*, indicating that the fitted equation should explain the variation of a dependent variable as a function of the independent variables, the results for the second and third steps (which theoretically should be the best) resulted in non-significant coefficients, and increased standard error for each coefficient. This increase may indicate the presence of a multicollinearity effect, demonstrated by the VIF values and the high linear correlations found between the independent variables in the third step.

Given the advantage based on the statistics of fit of the model obtained in the first step over the others and it successfully met all of the conditions required by the regression, the model obtained by the forward method was the same as that obtained by the stepwise method ($cv = b_1 d^2ch + \varepsilon i$).

Comparatively, the two equations show fits described in Table 1. The equations result in a variation of less than 1.5% in terms of $R^2_{adj}$, less than 5% with respect to $S_{yx}$ and similar distribution of residuals along the estimate curve. However, the *F* values for the equation obtained by stepwise and forward methods were more significant and yielded lower standard error in the coefficient. That is, the stepwise selection statistical procedure resulted in a more advantageous model than the backward method. These results corroborate those of Draper and Smith (1966), who assert that the stepwise method is one of the most recommended for the careful selection of explanatory variables in model construction.

In order to verify the performance of the best model, evaluations were conducted (in the same manner as that required for model generation) in 1, 3 and 5 year old plantations.

Chi-square values ($\chi^2$) were not significant for any of the ages, implying that there are significant differences between estimated and actual values. Furthermore, the statistical fits represented by $R^2_{adj}$ and $S_{yx}$ ranged from 86.01 to 88.63% and from 16.66 to 22.78%, respectively. These values were similar to those obtained from the fits used in model construction from data taken in seven year old stands, a fact that indicates stability in the model under different conditions. The fit also yielded significant *F* values, significant coefficients, low standard error for the coefficients, and adequate distribution of residuals over the whole estimate curve.

Meng et al. (2007) formulated models for crown volume of *Pinus contorta* Engelm. based on uniform stress theory and found that the models were able to represent about 70% of the variation in crown volume. In that study the independent variables were diameter at breast height, distance between the centre of the crown and the diameter at breast height, and wind speed. Davies and Pommerening (2008) concluded that models of crown volume of *Picea sitchensis* (Bong.) and *Betula* spp. using independent variables relating only to the bole were inefficient, especially in the case of suppressed trees. According to the authors, the inclusion of variables related to the spatial distribution of trees and stand structure considerably improved the models, representing up to 77% of the variation in the crown volume of the species.

By comparison, the models used in this study have showed statistical precision superior to those of the cited authors and have included fewer variables more appropriately selected. Such conditions characterize quality equations for estimation of the dependent variable, as defined by Draper and Smith (1966).

## Conclusions

The variable with the highest correlation with the

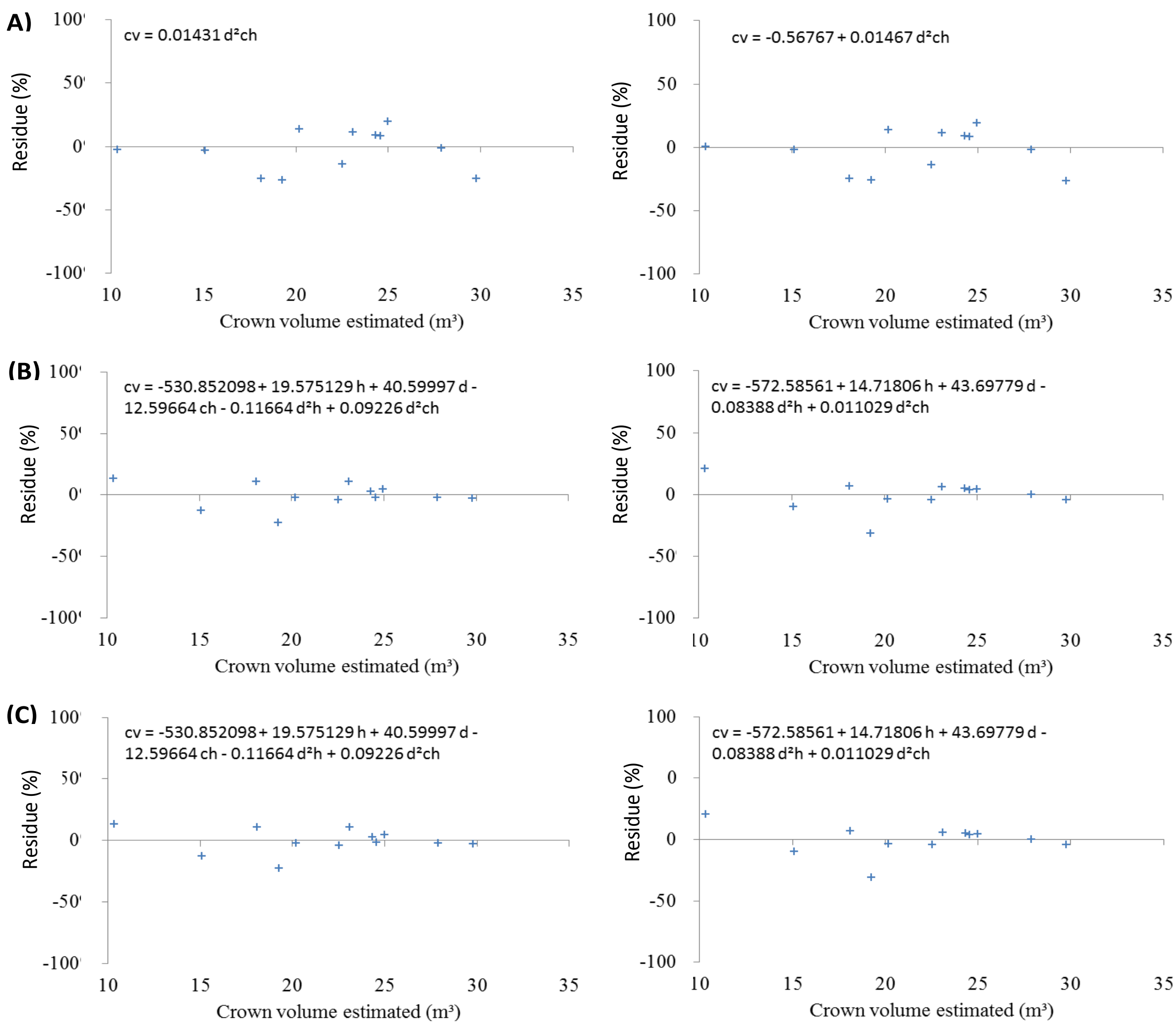


**Figure 1.** Distribution of residuals of crown volume with respect to estimated volume in stands of black wattle in Rio Grande do Sul, obtained from equations formulated by stepwise (A), backward (B) and forward (C) methods.

volume of the crown of black wattle was the squared diameter at breast height combined with the height of the crown (d²ch). This variable was therefore selected to compose the model that presented the best fit, resulting both from the stepwise as well as forward methods. The final model resulting from these methodologies (cv = $b_1$ d²ch + ε*i)* made it feasible to obtain crown volume both in aged rotation stands and in young stands, revealing stable statistical precision in both cases. In addition to the characteristics of accuracy, the obtained model includes easily measurable variables, and is therefore suited to activities requiring knowledge of the crown volume in black wattle.

## Conflict of interest

The authors declared that they have no conflict of interest.

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