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Effects of Different Planting Patterns on the Growth and Yield of Maize and Soybean in Northwest China

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Abstract

Aboveground and belowground interactions are crucial in the over-yielding of intercropping systems. However, the relative effects of aboveground and belowground interactions on yields in maize (*Zea mays* L.) and soybean (*Glycine max*) intercropping systems are still unclear. Field experiments, including measurements of plant height, soil-plant analysis development (SPAD) value, photosynthetically active radiation (PAR), root length density (RLD), root volume density (RVD), and grain yield, were conducted in 2018-2019 to analyze the advantages and effects of above-ground and belowground inter-species interactions. This study adopted three different planting patterns: mono-cropping maize (MM), mono-cropping soybeans (MS), and maize-soybean intercropping (IM and IS). This study showed that intercropping promotes the growth of maize and makes maize have a better photosynthetic environment, while the growth of intercropping soybeans is inhibited and the photosynthetic environment becomes worse. In the upper layer (0-40 cm) and close to the plants, the root growth and distribution of intercropped maize increased, resulting in greater root length density and volume density, while the root growth and distribution of intercropped soybean decreased, resulting in lower root length density and volume density. The intercropping increased the maize yield by 18.52-19.8%, and reduced the soybean yield by 55.87-57.44%. The results indicated that intercropping improves the competitiveness of maize and reduces the competitiveness of soybeans. The increase in maize yield made up for the loss of soybean yield and led to an overall significant advantage in the maize-soybean intercropping system.

Keywords: growth, intercropping, maize, PAR, root, soybean, SPAD, yield

1. Introduction

Intercropping is the practice of growing two or more crops in the same field for a significant part of their growing period (Li et al., 2020). A large number of studies have confirmed that reasonable intercropping can utilize above-ground light and heat resources and belowground water and nutrient resources at multiple levels (Fan et al., 2006), improve the utilization rate of natural resources, and result in high yield and stability of crop composite groups (Mei et al., 2012; Raphaël et al., 2010). The most commonly reported is the cereal-legume intercropping system; the most typical example is the intercropping of soybean and maize (Simbine et al., 2018). Maize and soybeans are important food crops in China, and maize-soybean intercropping is a common high-yield planting model in northern China. Due to the low economic benefits of soybeans, China's soybean planting area has been declining year by year (Yang et al., 2007) and the domestic market supply of soybeans mainly depends on imports. Therefore, while the maize planting area continues to increase, the development of the maize-soybean intercropping planting mode can benefit maize and soybean production.

The relationship between interspecific competition and complementarity is the core of productivity and yield improvement in intercropping systems. It is also the key to the successful operation of intercropping systems (Yin et al., 2020). The competition and complementarity for nutrients, water, and sunlight are the main factors often used to explain the advantages of intercropping. Zhang and Li (2003) reported that belowground interactions and rhizosphere effects between intercropped crops play an important role in the advantages of intercropping. Lv et al. (2014) found that the competition between two crops for nutrition is more important than the competition for sunlight in an intercropping system. Intercrop productivity may be more affected by belowground than

aboveground species interactions (Ghosh et al., 2009). The different planting patterns of intercropping can change the lighting environment of the system and the interaction on the ground to intercept more light. Mutual shading significantly affects intercropping productivity, because light plays an important role in photosynthesis and crop yield. (Midmore et al., 1988; Li et al., 2010). And the intercropping can influence the microclimate environment of interspecies, particularly the light transmission rate of crop groups (Yang et al., 2014).

The spatial distribution of roots and their density in the soil may determine the ability of a crop to acquire the nutrients and water necessary to sustain plant growth. Gao et al. (2010) indicated that the horizontal root growth of the crops was confined to the soil surface and the zone closest to plants. Ding et al. (2020) found that intercropping shallow-rooted pepper with deep-rooted alfalfa (*Medicago sativa* L.) can enhance root nutrient absorption in deep soil layers, increasing N use efficiency and thus reducing NO_3^- leaching. When the water absorption space and root distribution of intercropped components are different, this can produce a greater complementary effect compared to intercropped components with similar water absorption space and root distribution (Mu et al., 2013). However, in the maize-soybean intercropping system, the effect of the interaction between the aboveground and the belowground parts on the growth and development of crops in different growth periods has not been extensively researched. It is difficult to obtain relevant data on crop roots as the traditional soil drilling sampling method will cause damage to the root systems and other methods are frequently technically demanding and costly. Therefore, this study carried out related research on the spatio-temporal changes of roots in a maize-soybean intercropping system through studying the minirhizotrons, which allowed us to observe the complete roots, improved our work efficiency, and provided a certain theoretical basis for the development of maize-soybean intercropping in arid areas.

The objectives of this paper were to: (i) investigate the effect of intercropping system on the aboveground and underground parts of maize and soybean at different growth period, and (ii) analyze the effects of intercropping on the yield and yield composition factors of maize and soybean, and judge whether the maize-soybean intercropping has yield advantages.

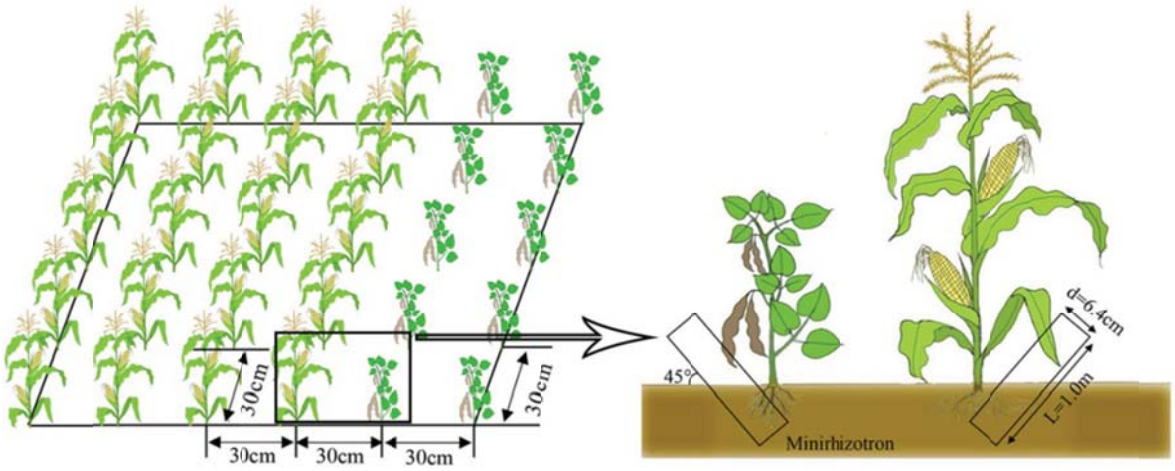
2. Materials and Methods

2.1 Study Site Descriptions

The maize cultivar ‘Jinnuo 205’ and the soybean cultivar ‘Xinda 1’ were used. Field experiments were conducted in 2018 and 2019 at a location in the Agricultural Experiment Station (45′08″N, 85′36″E) of Xinjiang Shihezi University, China. The mean temperature during the 2018 crop growing season (April-October) was 18.65 °C, the precipitation was 211.7 mm, and the sunshine duration was 2 077.3 h. The average temperature during the crop growing season (April-October) in 2019 was 20.94 °C, the precipitation was 142 mm, and the sunshine hours totaled 1 843.08 h. The soil texture was gray desert soil, the soil bulk density of the plough layer was 1.6 $g \cdot cm^{-3}$, the total nitrogen was 0.890 $g \cdot kg^{-1}$, the available phosphorus was 0.023 $g \cdot kg^{-1}$, the available potassium was 0.259 $g \cdot kg^{-1}$, the alkaline nitrogen was 0.058 $g \cdot kg^{-1}$, the organic matter was 13.260 $g \cdot kg^{-1}$, and the pH was 7.3.

2.2 Seeding Treatments

A two-year experiment was conducted with three treatments: (1) maize mono-cropping, (2) soybean mono-cropping, and (3) maize-soybean intercropping. The experimental was a randomized complete block design with three replications. The treatments were applied to the same plots in each year. The plant spacing and row spacing of maize and soybean was 30 cm. Four rows of maize were intercropped with 2 rows of soybean and the area of each test plot was 8.1 m^2(4.5 m × 1.8 m). The arrangement between rows is shown in Figure 1-I. Crops were sown on April 24, 2018 and April 26, 2019. The base fertilizer included 300 $kg \cdot hm^{-2}$ of diammonium phosphate, 75 $kg \cdot hm^{-2}$ of urea and potassium fertilizer, and 45 $kg \cdot hm^{-2}$ of urea in the maize spinning period. The experiment adopted the drip irrigation method. The irrigation frequency was once every seven days and the irrigation volume was 675 $m^3 \cdot hm^{-2}$ each time. The data collection time and the corresponding crop growth period are shown in (Table 1).



(I) (II)

Figure 1. Diagram of a maize-soybeans intercropping system (I) and the settings of Minirhizotron (II)

Table 1. Data measurement time and corresponding growth stages period of Maize and Soybean crops (2018-2019)

Stage	Date(2018-2019)	Growth stage period	
		Maize	Soybean
A	20/5, 21/5	Seedling	Seedling
B	2/6, 3/6	Jointing	Flowering
C	26/6, 28/6	Tassel	Flowering and podding
D	14/7, 17/7	Silking	Seed filling
E	25/7, 27/7	Milk	Mature
F	10/8, 11/8	Maturity	Harvest

2.3 Measurement Items and Calculations

The field photosynthetically active radiation was measured at 12 pm on June 26th, 2018 and June 28th, 2019. The LI-250A light meter was used to measure the photosynthetically active radiation in different parts of the crop under different planting modes. Monocropped and intercropped maize crops were measured at three sites: the upper, middle, and lower part of the plants. Monocropped and intercropped soybeans were measured at two sites in the upper part and the middle part. There were five replicates in the experiment, the chlorophyll meter can be used to measure the leaf SPAD value to predict the nitrogen nutrition status of the crop. The SPAD 502 chlorophyll meter was used to measure the relative chlorophyll content of plant leaves. The SPAD value was measured on the three leaves of maize on the cob and the top unfolded leaves of soybean. Each treatment was repeated with six replicates. Six plants were randomly selected for measurement in a monocropping plot.

CI-600 was used to collect images of the field root systems. During sowing, the transparent observation tube was buried in the crop row along the horizontal ground at 45° angle, and the root system of the crops in the first five periods was measured. Each treatment had three repetitions. The scanner collected the crop roots in the soil layers of 0-20 and 20-40 cm. WinRHIZO was used to analyze the root image and calculate the root length density, root volume density, and root surface area. The minirhizotron setting is shown in Figure 1-II.

Each treatment was harvested when the crops were mature. The harvest area was 4 m^2. Each treatment was repeated three times, and threshing and natural drying were performed uniformly. The grain yield was measured and converted into hectare yield. The land equivalent ratio (LER) was used as an index to measure the yield advantage of intercropping (Willey, 1979), and the calculation formula was:

$$LER = \frac{Yia}{Ysa} + \frac{Yib}{Ysb} \quad (1)$$

where, *Yia* represents the yield of intercropped maize, *Ysa* represents the yield of mono-cropping maize, *Yib* represents the yield of intercropped soybean, and *Ysb* represents the yield of mono-cropping soybean. If *LER* > 1,

it indicates that the maize-soybean intercropping system has yield advantage; if *LER* < 1, it indicates that the maize-soybean intercropping system has no yield advantage.

2.4 Statistical Analyses

All of the experimental data were managed by Microsoft Excel 2016 and the figures were constructed with Origin Pro 2018. Differences between intercropping systems and years were identified by analysis of variance (ANOVA) using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). The intercropping effect was estimated for each year individually because of significant year × treatment interactions for most of the variables assessed in the current study. The mean values were compared with a least significant difference (LSD) test at the ($P < 0.05$) significance level.

3. Results

3.1 Plant Heights

During the two years, the heights of maize and soybean plants increased as the growth period continued, and the plant heights of the crops tended to be stable in the later period of crop growth (Figure 2). Intercropping maize plant heights were significantly increased by 12.85-15.31%, 9.58-16.08%, 6.42-10.63%, and 5.77-11.33% in comparison with the mono-cropping crop at the tassel, spinning, milk maturity, and maturity stages, respectively in 2018 and 2019 ($P < 0.05$).

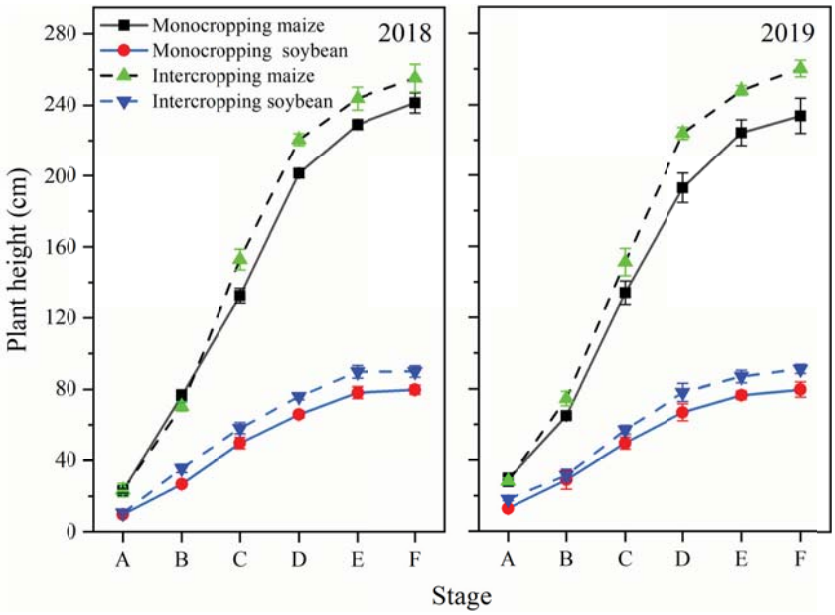


Figure 2. Dynamic changes of plant heights of maize and soybean under different planting patterns in 2018-2019 (n = 3)

The height of mono-cropping and intercropping soybean plants increased rapidly from the seedling stage to the seed filling stage during the two years. Intercropping soybean plant heights were significantly increased by 14.92-16.76%, 15.36-16.74%, 13.61-14.96%, and 12.79-14.46% in comparison with the monocropping crop at the flowering and podding, seed filling, mature, and harvest stages, respectively, in 2018 and 2019 ($P < 0.05$). No significant differences were found at the seedling and flowering stages in both experimental years.

3.2 Photosynthetic Characteristics of Crops

As shown in Figure 3, the soil-plant analysis development (SPAD) value of each treatment maize increased rapidly from the seedling stage to the jointing stage during the two years and reached the maximum during the jointing and tasseling stage, after which the SPAD value gradually decreased. The intercropping maize SPAD value was significantly increased by 14.87% in comparison with the monocropping crop in the milk maturity period in 2018 ($P < 0.05$), and the intercropping maize SPAD value was significantly increased by 9.15% in the tasseling period in 2019. There was no significant difference in SPAD values between monocropping and intercropping maize in other growth periods.

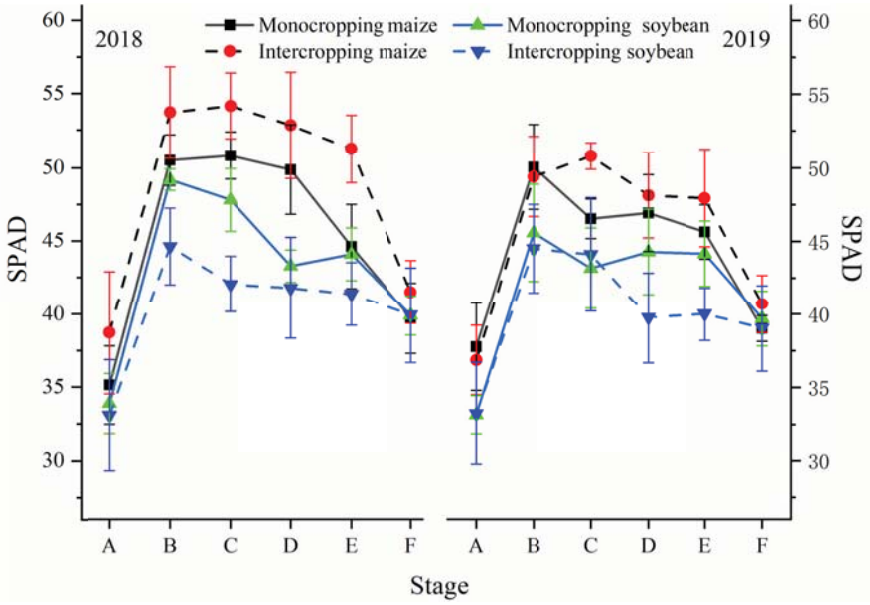


Figure 3. Dynamic changes of maize and soybean soil-plant analysis development (SPAD) values under different planting patterns in 2018-2019 (n = 3)

During the two years, the SPAD value of each soybean treatment increased rapidly from the seedling stage to the flowering stage and reached the maximum in the flowering stage in both years. The intercropping soybean SPAD values were significantly reduced by 9.27% and 12.00% in comparison with the monocropping crop in the flowering and pod stages in 2018 ($P < 0.05$), respectively. The intercropping soybean SPAD value was significantly reduced by 10.21% in comparison with the monocropping crop in the seed filling stage in 2019 ($P < 0.05$). There were no significant differences in SPAD values between monocropping and intercropping soybeans in other growth periods.

Different planting methods significantly affect the PAR between maize and soybean rows (Figure 4). The results of the two-year experiment showed that the maize-soybean intercropping significantly increased the photosynthetically active radiation in the lower and middle parts of maize by 9.63-11.33% and 22.78-36.10% in comparison with the mono-cropping crop in 2018 and 2019 ($P < 0.05$), respectively. However, intercropping significantly reduced the photosynthetically active radiation in the middle and upper parts of soybeans by 8.91-8.64% and 24.01-24.63% in comparison with the mono-cropping crop in 2018 and 2019 ($P < 0.05$) respectively.

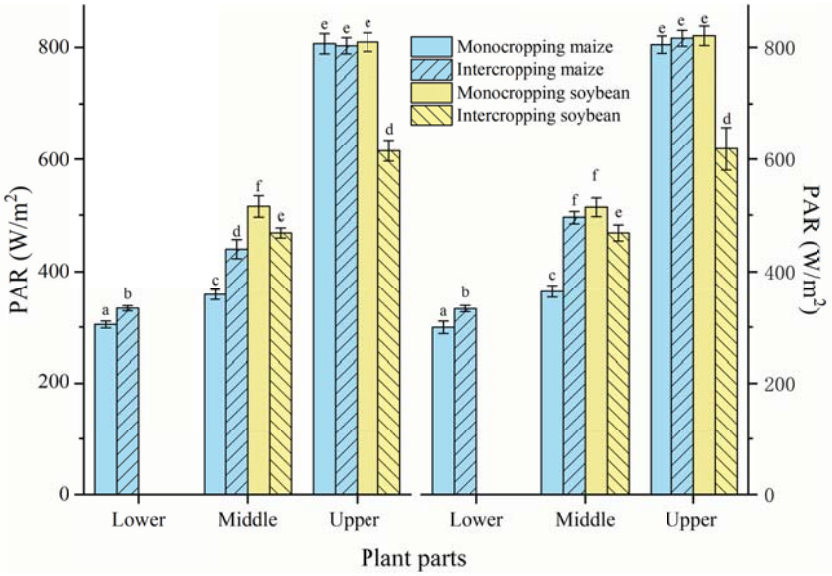


Figure 4. Photosynthetically active radiation (PAR) of different parts of crops under different planting modes

3.3 Root Growth and Distribution

With the advancement of the growth period of maize and soybean, the root length density (RLD) value gradually increased (Figure 5). At 0-20 cm depth, there were no significant differences in the RLD values of mono-cropping and intercropping maize between the growth periods of the crop in both 2018 and 2019 (Figure 5A). At 20-40 cm depth, the intercropped maize RLD value was significantly increased by 8.4 and 18.0% in comparison with the mono-cropping crop at the tasseling and spinning stages in 2018 respectively, and the maize RLD value was significantly increased by 22.6% at the tasseling stage in 2019 (Figure 5B). At 0-20 cm depth, the intercropped soybean RLD values were significantly reduced by 16.29-19.10% and 13.35-19.40% in comparison with the mono-cropping soybean at the seed filling and maturity stages respectively, in both 2018 and 2019 (Figure 5C). At 20-40cm depth, the intercropped soybean RLD value was significantly reduced by 17.34% in comparison with the mono-cropping soybean at the maturity stage in 2019. However, there were no significant differences between mono- and intercropped in each growth period in 2018 (Figure 5D).

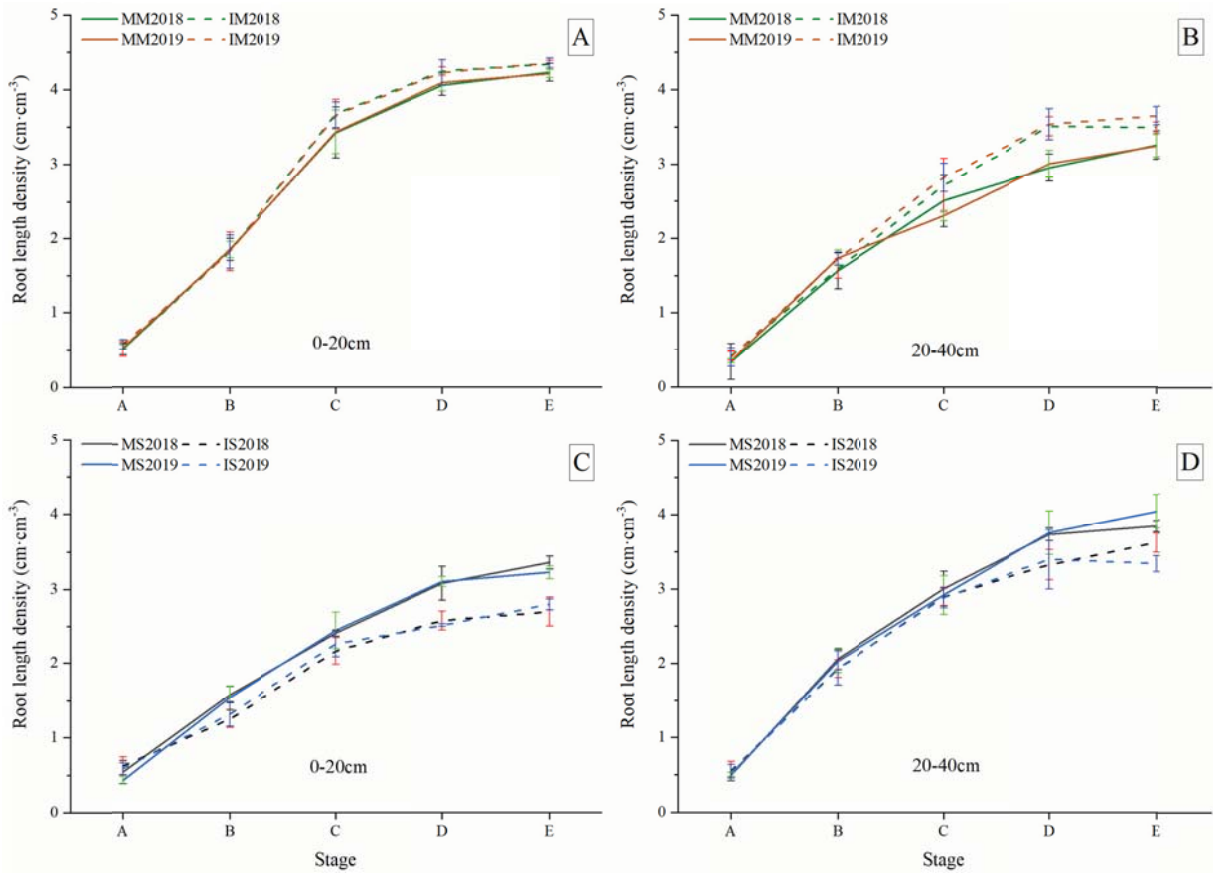


Figure 5. Dynamic change of root length density (RLD) value of soybean and maize in 0-40 cm soil layer under different planting patterns in 2018-2019 system in different planting modes

Note. MM: mono-cropping maize; MS: mono-cropping soybeans ; IM and IS: maize-soybean intercropping. A: Seedling stage of maize and soybean; B: Jointing stage of maize and flowering stage of soybean; C: Tasseling stage of maize and flowering and podding stage of soybean; D: Spinning stage of maize and seed filling stage of soybean; E: Milk maturity of maize and mature stage of soybean.

As the growth period of maize and soybeans advanced, the root volume density (RVD) value showed a single peak curve that first increased and then decreased (Figure 6). The greatest differences between mono-cropping and intercropping maize RVD values at each soil depth were as follows: at 0-20 cm depth, the maximum RVD values of mono-cropping and intercropping maize appeared at the spinning stage, and the intercropped maize RVD values were significantly increased by 8.92-9.91%, 12.27-15.92%, and 18.65-22.04% in comparison with the mono-cropping crop at the flower pod, seed filling, and harvest stages, respectively, in 2018 and 2019 (Figure 6A). At 20-40 cm depth, the maximum RVD value of monocropping and intercropping maize appeared at the tasseling stage, and the intercropped maize RVD values were significantly increased by 7.08-9.87%, 27.79-36.49% and 27.89-37.33% in comparison with the mono-cropping crop at the flower pod, seed filling, and harvest stages respectively, in 2018 and 2019 (Figure 6B). There were no significant differences in other growth periods.

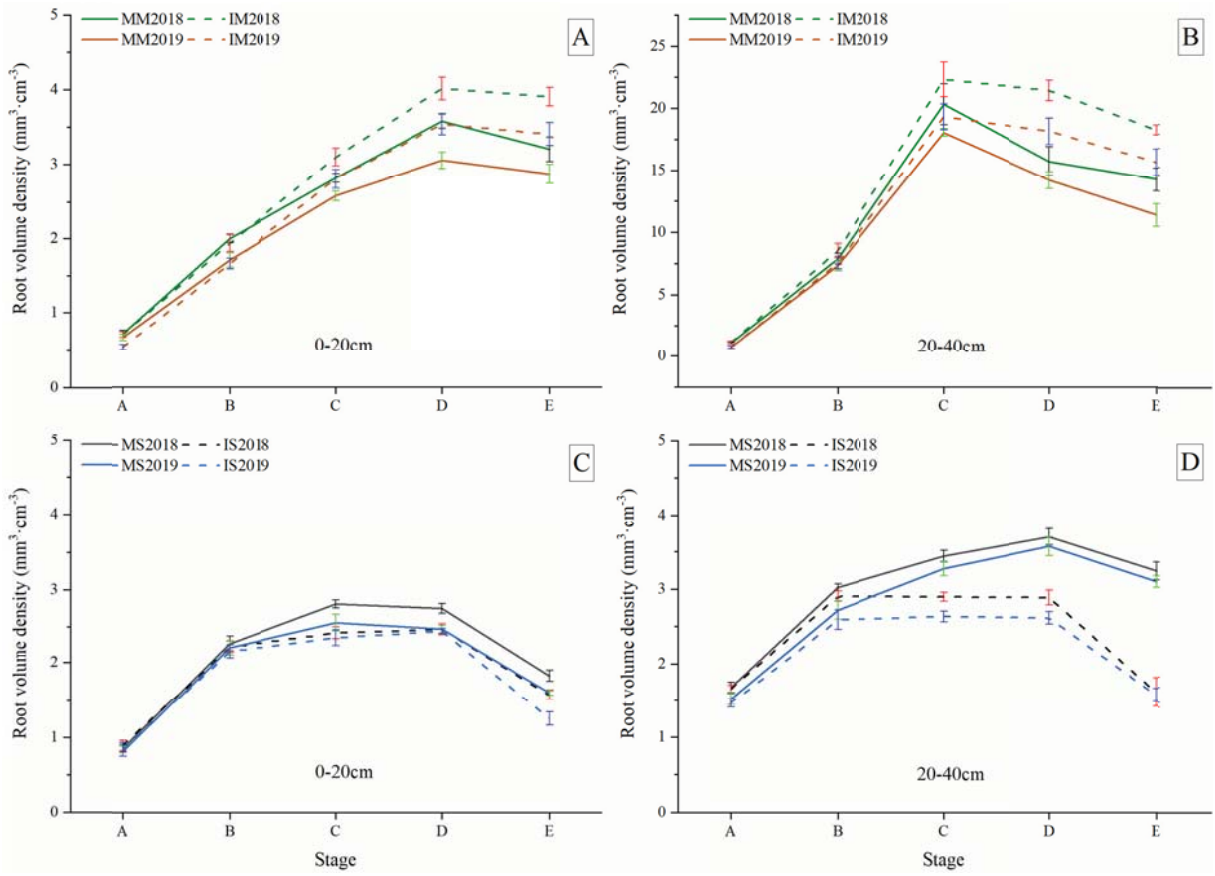


Figure 6. Dynamic change of root volume density (RVD) value of soybean and maize in 0-40 cm soil layer under different planting patterns in 2018-2019 system in different planting modes

Note. MM: mono-cropping maize; MS: mono-cropping soybeans ; IM and IS: maize-soybean intercropping. A: Seedling stage of maize and soybean; B: Jointing stage of maize and flowering stage of soybean; C: Tasseling stage of maize and flowering and podding stage of soybean; D: Spinning stage of maize and seed filling stage of soybean; E: Milk maturity of maize and mature stage of soybean.

The differences between mono-cropping and intercropping soybean RVD values at each soil depth were as follows: at 0-20 cm depth, the maximum RVD value of monocropping and intercropping soybeans appeared at the flower pod stage, and the intercropped soybean RVD values were significantly increased by 10.17-13.77%, 1.06-14.19%, and 7.93-22.29% in comparison with the mono-cropping crop at the flower pod, seed filling, and harvest stages in 2018 and 2019 (Figure 6C). At 20-40 cm depth, the maximum RVD value of mono-cropping and intercropping soybeans appeared at the seed filling stage, and the intercropped soybean RVD values were significantly increased by 15.80-19.56%, 21.97-26.82%, and 49.08-50.20% in comparison with the mono-cropping crop at the flowering and podding, seed filling, and mature stages respectively, in 2018 and 2019 (Figure 6D). There were no significant differences in other growth periods.

3.4 System Yield and Yield Composition Factors

Crop yield was significantly affected by planting methods, while the year effect and the effect of its interaction with planting patterns were not significant (Table 2). The most significant differences between maize yield and yield composition factors under mono-cropping and intercropping were as follows: intercropped maize 1000 grain weight and yield were significantly increased by 7.73-8.48% and 18.52-19.8% in comparison with the mono-cropping crop in 2018 and 2019 respectively. There was no significant difference in the panicle number per plant and the number of grains per spike between mono-cropping and intercropping maize in the two years. In relation to soybean yield and yield composition factors the most significant differences under mono-cropping and intercropping were as follows: There was no significant difference in the number of seeds per pod in mono-cropping and intercropping soybeans during the two years of experiments. However the intercropped soybean number of pods per plant, 1000-grain weight, and yield were significantly reduced by 34.51-35.45%,

13.34-16.45%, and 55.87-57.44% in comparison with the mono-cropping crop in 2018 and 2019, respectively. The two-year intercropping land equivalent ratio (LER) was calculated as 1.61-1.64 > 1 by Equation (1) (Willey, 1979), indicating that the intercropping system was advantageous.

Table 2. Crop yield and yield composition factors under different planting patterns in 2018-2019

Years	Treatment	No. of panicle·plant^{-1} No. of pod·Plant^{-1}	No. of seeds·spike^{-1} No. of seeds·pod^{-1}	1000 grain weight (g)	Yield (kg·hm^{-2})
2018	Mono-cropping maize	1.67±0.47 a	188±5.72 a	404.33±3.40 a	4969.41±127.68 a
	Intercropping maize	1.67±0.47 a	195±5.35 a	438.60±1.72 b	5953.36±130.54 b
2019	Idem	1.33±0.47 a	188.33±1.25 a	407.1±1.96 a	4950.3±115.49 a
	Idem	1.67±0.47 a	195±1.63 a	438.57±3.96 b	5866.95±123.84 b
2018	Mono-cropping soybean	36.67±2.05 b	2.67±0.47 a	242.6±7.03 a	1457.27±82.90 a
	Intercropping soybean	23.67±3.30 a	2.67±0.47 a	202.7±1.79 b	643.11±49.29 b
2019	Idem	37.67±1.7 b	2.67±0.47 a	234.95±2.08 a	1525.82±43.41 a
	Idem	24.67±2.05 a	2.33±0.47 a	203.6±4.28 b	649.34±67.77 b

3.5 Correlation Between Different Indicators of Crop Growth Stages and Yield

The Pearson correlation coefficient was obtained through correlation analysis of crop height, SPAD, RLD, RVD value and crop yield under different growth periods (Table 3). Throughout the growth period of the crop, the yield of maize was positively correlated with plant height, SPAD, RLD and RVD value. Soybean yield is negatively correlated with plant height, and positively correlated with other indicators. And the indicators that are significantly related to crop yield mostly occur in the middle and late stages of crop growth. Indexes that are highly correlated with maize yield are plant height and RLD, and indexes that are relatively correlated with soybean yield are plant height, RLD and RVD value.

Table 3. Pearson correlation coefficient between different indicators of crop growth stages and yield

Crops	Index	Stage					
		A	B	C	D	E	F
Maize	Plant height	-0.063	0.176	0.995**	0.958*	0.959*	0.937
	SPAD	0.548	0.441	0.72	0.509	0.905	0.929
	RLD (0-20cm)	0.367	-0.978*	0.997**	0.987*	0.985*	
	(20-40cm)	0.989*	0.037	0.894	0.994**	0.922	
	RVD (0-20cm)	-0.444	-0.148	0.718	0.699	0.848	
	(20-40cm)	0.125	0.533	0.559	0.906	0.849	
Soybean	Plant height	-0.445	-0.854	-0.994**	-0.984*	-0.982*	-0.996**
	SPAD	0.51	0.7	0.513	0.89	0.959*	0.435
	RLD (0-20cm)	0.989*	0.037	0.894	0.994**	0.922	
	(20-0cm)	-0.923	-0.972	0.715	0.992**	0.895	
	RVD (0-20cm)	-0.61	0.573	0.82	0.57	0.715	
	(20-40cm)	0.16	0.338	0.921	0.965*	0.992**	

Note. A: Seedling stage of maize and soybean; B: Jointing stage of maize and flowering stage of soybean; C: Tasseling stage of maize and flowering and podding stage of soybean; D: Spinning stage of maize and seed filling stage of soybean; E: Milk maturity of maize and mature stage of soybean; F: Maturity stage of maize and harvest stage of soybean.

* Significant at 5% ($P < 0.05$); ** Significant at 1% ($P < 0.01$).

4. Discussion

The intercropping of maize and soybeans is a weak competition system. Interspecific facilitation in maize-soybean intercrops may be due to increased efficiency of resource use (Hamel et al., 1991) and the compensatory distribution of root systems (Gao et al., 2010). To reduce interspecific competition, intercropped species are

separated in the time of absorption and utilization of water, nutrients, and other resources and there are differences in the utilization of resources in different niches, resulting in intercropping advantages (Xia et al., 2013).

The results of this study indicated that intercropping promotes the height of maize and soybean, and the plant height of maize is positively correlated with yield, while the plant height of soybean is negatively correlated with yield. In intercropping systems, shorter crops suffer shading from taller crops, thus increasing plant height and decreasing yield (Wu et al., 2016).

In the early stage of crop growth, the crop is in the vegetative growth stage, and the biomass is supplied to the roots, stems and leaves of the crop. In the middle and late stages of the crop growth, the vegetative growth transitions to reproductive growth, so that the biomass is supplied to the grains and the growth rate of the plant height decreases (Na et al., 2018). In addition, in the early stage of soybean growth, since the degree of shading from maize plants to soybean plants was not obvious, there was no significant difference in the height of soybeans under monocropping and intercropping modes. As the growth period advanced, the degree of shade from maize to soybeans increased and the taller maize gained a competitive advantage in the maize-soybean intercropping system. As a result, the shaded soybeans intercepted less incident radiation. In full sunlight, the proportion of biomass distributed to the leaves was significantly higher than the petioles and stems. However, under shade, the proportion of biomass distributed to the leaves was lower than the stems. Soybean showed a significant stem elongation response under shaded, leading to the production of less biomass and reduced grain yield (Wu et al., 2017).

The chlorophyll content of crop leaves is closely related to nitrogen content and the chlorophyll meter reading is positively related to the chlorophyll content (John, 1983; Hallik, et al., 2009). Nitrogen (N) is critical for the growth and development of crop plants. While most plant species depend on the uptake of soil N to satisfy their needs, certain clades, most notably the legumes, are capable of fixing N via a symbiotic relationship with rhizobia bacteria (Carranca, 2013). This fixed N may benefit not only the legumes but also companion/subsequent crops. The results of this study indicated that the SPAD values of maize and soybean increased rapidly and reached the maximum in the early growth period, after which the SPAD values of crops gradually decreased (Figure 3). This was due to the transition of crops from vegetative growth to reproductive growth and nitrogen transfers from vegetative organs such as leaves to reproductive organs such as grains, resulting in a decrease in the SPAD value of leaves (Wang et al., 2014). Compared with monocropping, intercropping increased the SPAD value of maize and decreased the SPAD value of soybean. As the degree of shade on soybeans was higher, the SPAD value was lower (Liu et al., 2016).

PAR refers to the solar radiation spectrum with a wavelength of 400-700 nm, which can be absorbed by green plant leaves and used for photosynthesis (Mizoguchi et al., 2014). Previous studies demonstrated that photosynthesis is the basis for yield formation, and 90% of dry matter comes from photosynthesis (Gaju et al., 2016). Changes in PAR are related to the effects of species, varieties, planting methods, and row spacing. The planting patterns of maize-soybean intercropping systems can induce changes in the microclimate environment, particularly in the light intensity and the spectral properties of the soybean canopy with its lower layer (Awal et al., 2006). The different planting patterns of intercropping may change the light environment of the system and the interaction on the ground to intercept more light, and mutual shading significantly affects the productivity of intercropping (Midmore et al., 1988; Cao, 2010). The results of this study indicated that compared with monocropping, intercropping significantly increased the PAR in the lower and middle parts of maize, and the PAR of the soybean in monocropping planting and intercropping varied significantly because the incident light reflected and absorbed by maize leaves reduced the amount of incoming PAR that was available for the soybean seedlings in intercropping conditions, resulting in a decrease in PAR in the middle and upper intercropping soybeans (Figure 4). Similarly, Lv et al. (2014) reported that taller maize plants in an intercropping system affect the light environment of a shorter species, such as soybean.

The crop growth and final yield of an intercropping system are closely related to the spread of roots, which determines the uptake and utilization of water and nutrients. Maize has a stronger resource competitive advantage than soybean. Intercropping can expand the vertical and horizontal spatial niche of crop roots, that is, expand the crop's water and nutrient niche, and increase the effective space for crops to absorb nutrients (Yong et al., 2012). Gao et al. (2010) reported that the roots of the two are mainly distributed in the 0-30 cm soil layer during the intercropping of maize and soybeans. This study showed that the RLD value difference between monocropping and intercropping maize began to increase gradually after the jointing stage. The growth of roots in the 20-40 cm soil layer of intercropped maize expanded the vertical niche of the maize root system. Yong et al. (2018) showed that the intercropping of maize and beans expanded the niche of the maize root system in the horizontal and vertical directions, and the root length density and root surface area were positively correlated with nitrogen absorption. In the intercropping system of maize and soybeans, intercropping soybeans promoted the growth of

maize through nitrogen fixation. Nitrogen uptake promotes the vigor of maize root systems (Bethlenfalvay et al., 1991). After the flowering and pod stage, the difference in RLD value of monocropping and intercropping soybeans began to gradually increase. Intercropping inhibited the growth of soybean roots in the 0-40 cm soil layer, and the root system in the 0-20 cm soil layer was more inhibited, making the soybean roots spread horizontally. From the RVD value, can be found that in the 0-40 cm soil layer, there was no significant difference in the RVD value of crops in the early stage of growth between monocropping and intercropping, while in the middle and late stages of growth, the RVD values of intercropped maize were significantly increased and intercropped soybean RVD values were significantly reduced in comparison with the monocropping crop. In the later stage of crop growth, due to the gradual decrease of crop irrigation and the transition from vegetative growth to reproductive growth of crops, the biomass of crop roots gradually decreases, resulting in a decrease in root volume density. Yin et al. (2014) reported that the growth of intercropped soybeans was significantly inhibited at the seedling stage, which is inconsistent with the results of this study. This study suggested that in the middle and late stages of crop growth, soybean photosynthesis preferentially supplies stem elongation, which seriously affects and inhibits the growth of the underground part of soybean plants.

The yield of cereal crops is determined by the number of panicles, grain number per spike and grain weight. The coordinated development of these three yield components is an important basis for obtaining high yields. Anderson et al. (1986) and Krarup and Davis (1970) proposed that leguminous crops yield could be expressed as the product of number of pods, seeds per pod and seeds weight. Lesoing et al. (1999)reported that maize yields were increased compared with inside rows in strip-intercrop soybeans of varying plant heights when intercropped. Shading of the legume was also reported for reducing seeds weight in legumes both in intercrop and sole crop as seeds filling becomes source limited if photosynthesis is reduced (Neugschwandtner & Kaul, 2014). Monti et al. (2016) reported that the intercropping system affected the pod setting stages in pea causing a large reduction in number of pods per plant and pea grain. This study showed that intercropping increases the thousand-grain weight of maize, thereby increasing the yield of intercropping maize. Intercropping reduces the number of pods·plant^{-1} and the thousand-seed weight of soybeans, resulting in lower soybean yields. This study believes that the main reason for the reduction of soybean production by about 50-60% is that the shading of soybeans by maize will reduce the formation of soybean photosynthesis, and the lack of nutrients required for the growth of flowers and pods has led to the fall of soybean flowers and pods, which seriously affected soybean production. Pearson correlation analysis shows that the main factors of crop yield formation are mostly distributed in the middle and late stages of crop growth. And the LER of the maize-soybean intercropping was 1.61-1.64, indicating that maize and soybean intercropping had planting advantages.

5. Conclusions

The aim of this study was to gain an understanding of how intercropping agronomic practices will affect the growth and yield of maize and soybean in Northwest China. Our results show that the intercropping system makes the maize form a better photosynthetic environment, and promotes the growth of the aboveground and deep root system of maize. The photosynthetic environment of intercropping soybeans becomes more severe, which inhibits the growth of soybeans and the growth of soybean roots. The intercropping system increases the weight of maize kernels and increases the yield of maize, while the intercropping system affects soybean pod formation, resulting in a large reduction in the number of pods per plant, and reducing the thousand-seed weight of soybeans, resulting in a decrease in soybean yield. In 2018 and 2019, the land equivalent ratio was 1.61 and 1.64, the benefits are significant. And improved field management in the middle and late stages of crop growth may further increase the productivity of the intercropping system.

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Effect of Inoculum Concentration and Pretreatment on Biomethane Recovery From Cotton Gin Trash

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Abstract

The potential of cotton gin waste, a considerable challenge to the gin owners, has not been fully investigated as a renewable energy source via anaerobic digestion. The weathered cotton gin trash and inoculum for triplicate biomethane potential assays were obtained from a local cotton gin mill and a municipal wastewater treatment plant, respectively. The moisture, total solids, volatile solids, and C, H, N, S, hemicellulose + cellulose, and lignin contents of gin waste were determined in triplicates. The biomethane potential of untreated and pretreated (hot water and 6% NaOH (wet CGT weight basis) gin waste was determined at different inoculum to substrate ratios. The highest cumulative biomethane yield of 111.8 mL g_{vs}^{-1} was observed in inoculum to substrate ratio of 2.3, and it was statistically similar to the values; 101.8, 104.7, 100.5, and 108.9 g_{vs}^{-1}, observed in 0.8, 1.2, 1.5, and 1.9, respectively. The biomethane yield at the inoculum to substrate ratio of 0.4 was significantly lower than all higher ratios. The $T_{80\text{-}90}$ for biomethane production was 26-30 for the ISRs of 1.2, 1.5, and 2.3. The $T_{80\text{-}90}$ for inoculum to substrate ratios of 0.4, 0.8, and 1.9 were 26-31, 27-32, and 27-31 d, respectively. The modified Gompertz equation fitted very well (R^2 = 0.98-0.99) to the anaerobic digestion at all inoculum to substrate ratios and pretreatments as the observed and predicted biomethane values were similar. The model predicted a lag phase of 8-10 days for control and treatments compared to the observed of 10-15 days. The highest biodegradability of 24.8±2.6% was observed at inoculum to substrate ratio of 2.3, which was statistically similar to the values observed in ratios of 0.8, 1.2, 1.5, and 1.9, respectively. Among pretreatments, the highest biodegradability of 33.0±2.4 was observed in 6% NaOH pretreatment, and it was statistically similar to hot water treatment and non-pretreated or control. These research findings advance the knowledge in the anaerobic degradation of cotton gin trash, thus helping to maximize biomethane recovery from this agro-industrial waste.

Keywords: anaerobic digestion, inoculum to substrate ratio, biomethane potential assays, kinetic modeling, pretreatment

1. Introduction

Cotton is one of the world's most important cultivated crops owing to its high-quality natural fiber. According to a recent statistical report, around 26.7 million metric tons of cotton were produced globally during the 2019-20 growing season, and the United States was the major exporter (Cotton Incorporated, 2020). Texas is a major cotton-producing state in the US; other US states producing cotton are Georgia, Arkansas, and Mississippi (Figures 1 and 2). The upland cotton (*Gossypium hirsutum*) is dominant throughout Texas. Pima cotton (*G. barbadense*), which is well adapted to the desert, is grown in far western Texas.

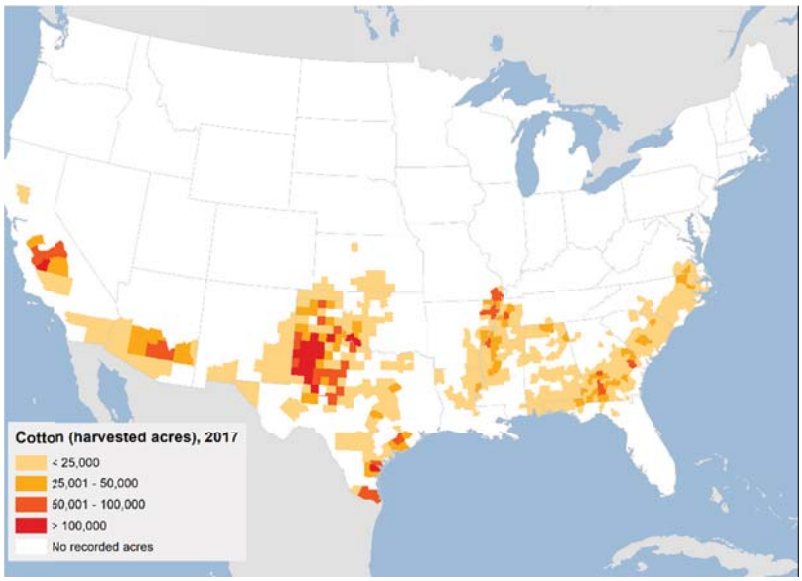


Figure 1. Cotton harvested acres, 2019

Source; USDA.

Once picked from the farm, cotton is further processed in gins to separate fiber, leaving behind cotton gin trash (CGT) as agro-industrial waste. According to Thomasson (1990), ginning one bale of cotton yields about 37-147 Kg of CGT, thus annually generating millions of tons of CGT in major cotton growing US states (Figures 1 and 2). The composition and amount of CGT generated depend upon the cotton cultivar and harvest method (Agblevor, 2006; Thomasson, 1990). In the US, cotton is mechanically harvested, and it is mainly composed of clean lint (8-15%), hulls (18.5-32.9), stem (5.2-5.9), grass (0.1-1.1), seed (0-2.9%), motes (20.4-21.6%), small leaves (19.4-34.9%) (Agblevor, 2003). The traditional management strategies have been to use it for feeding and bedding for the animals or composting, followed by addition to the soil as humus. The presence of microbial pests (verticillium wilt in particular) and pesticide residues, low heating value (Holt, 2006) in energy production limit the recycling options of CGT (Hamawand et al., 2016; Haque et al., 2020). The proper disposal of CGT is a challenge for the gin mills; thus, the gin mill owners spend money on disposing of CGT (Thomasson, 1990; Hamawand et al., 2016). The other potential uses of CGT include as a source of micro and nanocrystalline cellulose, dye absorbent in wastewater treatment, and a component of transparent plastics (Haque et al., 2020).

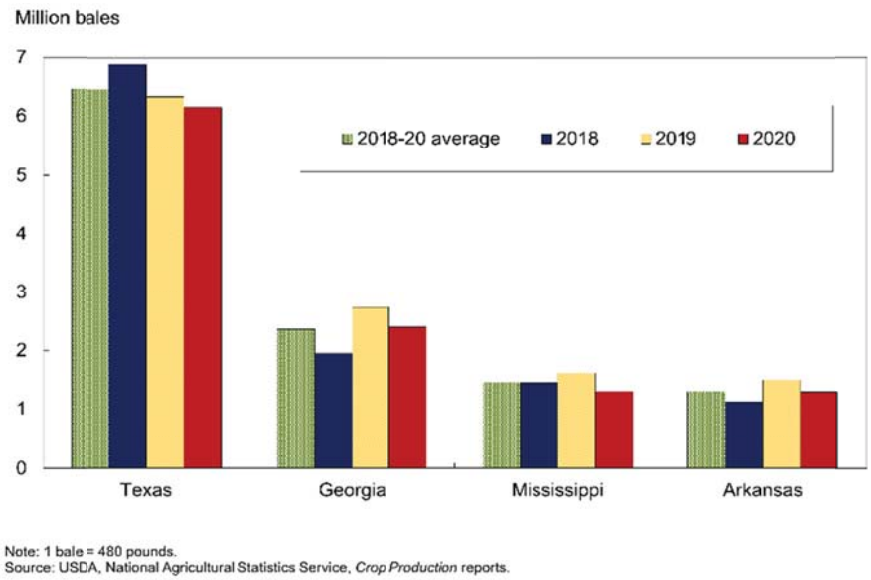


Figure 2. Leading US cotton-producing states, 2018-2020

However, the urgency to lower fossil fuel consumption had the scientists investigating agro-industrial wastes such as CGT as potential renewable energy sources (Zabaniotou et al., 2010). Consequently, CGT has been investigated as a bioenergy source through ethanol production, gasification, and anaerobic degradation (Hamawand et al., 2016). The high chemical oxygen demand (COD) and volatile solid (VS) contents in CGT can be recovered as biomethane through anaerobic degradation (Hamawand et al., 2016; Wilde et al., 2010).

Anaerobic degradation is widely adopted to convert organic matter into biomethane (USEPA, 2019; Guo et al., 2015). According to the United States Environmental Protection Agency (USEPA), as of October 2019, 287 anaerobic digesters were operating at the animal farms across the nation (USEPA, 2019). They were adopted by the farmers to curb methane emissions from animal farms (manures) as directed by EPA under the regulation of the National Pollutant Discharge Elimination System (USEPA, 2019). Biomethane potential (BMP) assays are used to determine a given substrate's suitability for anaerobic digestion. Anaerobic digestion relies upon the intricate balance of various bacterial groups which carry out the four distinct phases of hydrolysis (substrate break down to simple organic and amino acids), acidogenesis (conversion of simple organic and amino acids to volatile fatty acids, H_2 and CO_2), acetogenesis (volatile fatty acids are converted to CH_3COOH) and finally methanogenesis (Meegoda et al., 2018). The microbial degradation is impacted by inoculum, substrate, experimental and operational conditions (Raposo et al., 2011). Some of the most important factors impacting the process; hence biomethane yield are inoculum to substrate ratio or ISR and substrate composition (Ntiamoah-Ohemeng & Datta, 2019; Raposo et al., 2011).

There are some studies (Cheng & Zhong, 2014; Adl et al., 2012; Isci & Demirer, 2007; Funk et al., 2005) that focused on the BMP and/or ISR of cotton wastes in mono-digestion or co-digestion with manures. Adl et al. (2012) determined the effects of pretreatment, inoculum source, and feed to inoculum ratio (F/I) on the BMP of cotton stalks. Cheng and Zhong (2014) investigated the effects of the F/I, pretreatment, and co-digestion (with swine manure) on the BMP of cotton stalks. Additionally, the authors fitted the modified Gompertz equation to the cumulative experimental data and reported a high correlation between experimental and predicted values. Funk et al. (2005) co-digested CGT with swine manure (the mixing ratios from 1:1-10:1) in a two-stage bioreactor. Isci and Demirer (2007) reported that the addition of basal media yields higher biomethane during the anaerobic digestion of cotton oil cake, seed hull, and stalks.

Once the particle size and crystallinity of the biomass are reduced via mechanical milling, several pretreatment methods are available to remove the lignin fraction of the biomass, as summarized by Hassan et al. (2018). Two of these methods are hot water and alkaline treatments, which remove soluble and whole lignin and hemicellulose fractions, respectively (Hassan et al., 2018). The effect of these pretreatments on BMP of CGT still has not been investigated.

The biomethane potential assays were conducted at different inoculum to substrate ratios utilizing untreated and pretreated (hot water and alkali) cotton gin trash as feedstock. The digester performance at each treatment was evaluated by simulating the process with mainstream mathematical models and comparing them with theoretical values.

2. Method

2.1 Substrate and Inoculum

The aged CGT (from more than 3 months cotton ginning, Figure 3) was collected from Varisco-Court Gin Co. (5354 Steel Store Rd, Bryan, TX, 77807). The samples were collected from weathered, transition, and core layers and mixed to obtain a uniform gin waste. It was passed through Willy mill, sieved (2 mm) to obtain small particles, and stored at 4 °C in the refrigerator.

Figure 3. Cotton gin trash

The fresh inoculum obtained from the Prairie View wastewater treatment plant (operating at mesophilic temperature) was de-gassed (Holliger et al., 2016; Angelidaki et al., 2009) for a week and stored at 4 °C. The well-mixed samples of CGT and inoculum in triplicates were characterized for VS per method 2540 (APHA, 2005) using a Lindberg Blue M Electric furnace (Fisher Scientific, Pittsburgh, PA). The elemental composition (C, H, N, and S) of the CGT was determined with the Flash 2000 elemental analyzer (Elementar Americas Inc., Ronkonkoma, NY). The lignin, cellulose, and hemicellulose contents were determined by thermogravimetric analysis in the Perkin Elmer Diamond TG/DTG system.

2.2 Biomethane Potential Assays

2.2.1 Effect of Inoculum to Substrate Ratio

The effect of ISR on the biomethane recovery from the CGT during anaerobic degradation was determined by running BMP assays in 250 mL serum bottles (Fisher Scientific, Pittsburgh, PA). Of the twenty-one bioreactors, each containing 1.3 g CGT (1.0 g_{vs}), the control bioreactors did not receive inoculum (0 ISR). The rest of them received varying amounts of inoculum (0.5, 1.0, 1.5, 2.0, 2.5 and 5 mL) to obtain ISRs of 0.4, 0.8, 1.2, 1.5, 1.9 and 2.3, respectively. Blank bioreactors contained all ingredients except CGT. An equal volume of trace medium (Moody et al., 2009) was added to each bioreactor. The working volume of 100 mL in each bioreactor was achieved by adding deionized water. At day zero, the contents of each bioreactor were analyzed for pH, which varied between 6.5 and 7.8, which is in the optimum range (Filer et al., 2019) at the start of the assays. The bioreactors were purged with N_2 gas (99.9% purity) for five minutes and sealed with straight plug stoppers, and secured using aluminum crimp seals (Fisher Scientific, Pittsburgh, PA) for biomethane sampling and maintaining the anaerobic conditions, respectively. The bioreactors were incubated at 36±1 °C, and biomethane was measured (liquid displacement method, by using alkaline water, pH 10.30) daily. The daily gas measurements were continued until the total biomethane production during three consecutive days was less than 1% of the previously accumulated biomethane (Holliger et al., 2016).

2.2.2 Effect of Pretreatments

The effect of hot water and alkali pretreatments on the BMP of CGT was determined as described by Adl et al. (2012) and Pang et al. (2008), respectively. Triplicates of 10 g CGT (7.9 g_{vs}) were subjected to thermal or 0.6% NaOH in 250 mL serum bottles. Briefly, 50 mL DI water was added to all serum bottles. For thermal treatment, serum bottles were sealed using plastic caps and aluminum cramps and placed in a water bath. The temperature was gradually raised to 100 °C and maintained for 15 min. The serum bottles were brought to room temperature before the addition of the remaining ingredients.

For alkali treatment, 0.6 g or 6% NaOH (CGT wet weight basis) was directly added to each of the bottles. Triplicate blanks received all ingredients except CGT. The BMP assays were run with an ISR of 2. Other experimental conditions were the same as described in section 2.2.1.

2.3 Theoretical Maximum Biomethane Yield

The theoretical maximum biomethane yield (451.0 mL g_{vs}^{-1}) was determined by Buswell's equation (Buswell & Neave, 1930) below as described by Raposo et al. (2011) from the empirical formula of CGT ($C_{390.0}H_{450.0}O_{290.8}N_{21.2}S_{0.6}$) using the results of elemental analysis (Table 1).

$$TMY = \frac{22400 \times \left[\left(\frac{a}{2}\right) + \left(\frac{b}{8}\right) - \left(\frac{c}{4}\right) - \left(\frac{3d}{8}\right) - \left(\frac{e}{4}\right)\right]}{12a + b + 16c + 14d + 32e} \quad (1)$$

Where, a = 390.0, b = 450.0, c = 290.8, d = 21.2, e = 0.6.

2.4 Biodegradability

The biodegradability (BD) of a substrate is its fraction that is converted to biomethane during anaerobic digestion. The BD of CGT was determined using cumulative biomethane yield (EBY) from the experimental and theoretical biomethane (EMY and TBY) as described by Raposo et al. (2011).

$$BD\ (\%) = \frac{EBY}{TBY} \times 100 \quad (2)$$

2.5 Biomethane Production Kinetics

The anaerobic degradation process or bacterial growth in the bioreactors can be described by fitting the modified Gompertz equation developed by Lay et al. (1996, 1997) as described below:

$$P(t) = P_0 \times \exp\left\{-\exp\left[\frac{R_m \cdot e}{P_0}(\lambda - t) + 1\right]\right\} \quad (3)$$

Where, P(t) = The cumulated methane (mL g_{vs}^{-1}, minus the blank) at digestion time t (days); P_0 = Maximum cumulative methane production (mL g_{vs}^{-1}); R_m = Maximum daily rate of biomethane production (mL g_{vs}^{-1} d^{-1}); λ = lag phase (days), minimum time to produce biomethane; e = Mathematical constant 2.718.

2.6 Data Analysis

The experimental data were processed in Microsoft Excel 2010 (Microsoft, USA). The biomethane volume was converted to dry gas volume at STP by multiplying with a dry biomethane factor of 0.838 as described by Richards et al. (1991). The blank value was subtracted from the treatment values. All of the data were analyzed using the general linear model (GLM) and analysis of variance procedure of Statistical Analysis System (SAS® 9.2, SAS Institute Inc., Cary, NC, USA), and statistically significant treatment means were separated using the least significant difference (LSD) test at 5% probability.

3. Results and Discussion

3.1 Cotton Gin Trash Composition

Carbohydrates (hemicellulose + cellulose) were 56%, while lignin or acid-insoluble fraction was found to be 32.7% (Table 1).

Table 1. Cotton gin trash and inoculum characterization

Parameter (%)	Inoculum	CGT
Moisture	97.2±0.3	12.3±0.4
TS	2.8±0.3	87.7±0.4
VS	1.5±0	78.9±0.8
Ash	1.5±0.4	8.8±0.6
N	-	1.3±0
C	-	47.3±1.2
H	-	4.5±0
S	-	0.2±0
O	-	46.6±1.5
C/N	-	36.3±0.9
Cellulose + Hemi-cellulose	-	56.0
Lignin	-	32.7

Note. All values, except moisture, TS, VS, and ash are mean±SD from triplicate percentages of total sample dry weight basis (w/w).

Agblevor et al. (2006) reported 11.2, 37.1, and 21.7 to 27% ash, total carbohydrates, and acid-insoluble fractions in CGT collected from gin mills in the US. The CGT was collected from a pile that had been stored in the open in a local gin mill for 3 months.

The open storage of the CGT alters the composition of the biomass, lowering the total carbohydrate content (Agblevor, 2006). Although, the CGT for this study was collected from an outdoor storage pile, the carbohydrate content was similar to the fresh sample utilized by Jeoh and Agblevor (2000). The percentage of C, H, N, and S was 47.3, 4.5, 1.3, and 0.2, respectively. The value for oxygen was determined by subtracting the total sum of percent values of C, H, N, and S from 100. The observed C/N ratio of CGT; 36.3±0.9; is in the range of 28.0-51.3 in previous studies (Hamawand et al., 2016; Santos et al., 2016; Majglinao et al., 2015).

3.2 Daily Biomethane Yield

There was no effect of ISR on the total volume of biomethane on day one after incubation. On day 2, the bioreactors with inoculum had significantly higher biomethane volume as compared to the control (Figures 4 and 5). The peak volumes were observed between days 13-17. For 0 ISR, maximum biomethane of 1.5 mL g_{vs}^{-1} was observed on day 3 and stopped on day 14. For ISRs 0.4, 0.8, 1.9, and 2.3, the maximum daily biomethane volumes of 15.5, 14.7, 18.2, and 16.3 mL g_{vs}^{-1}, respectively, were observed on day 16. The biomethane volume from ISR 1.2 peaked at two consecutive days (16 and 17) at 13.3 and 13.4 mL g_{vs}^{-1}. Similarly, two peaks of daily biomethane volumes of 13.5 and 15.5 mL g_{vs}^{-1} were observed on days 13 and 19 for ISR of 1.5.

In CGT pretreatment assay, up to 12 d, lower daily biomethane values were observed in 6% NaOH than hot water and control. However, alkali-treated CGT consistently yielded higher biomethane from 13 to 27 d.

3.3 Cumulative Biomethane

From the cumulative biomethane chart (Figures 4 and 5), it was observed that without inoculum, CGT was not anaerobically degradable. For all ISRs, there was a lag phase of 10 days, indicating hydrolysis as the rate-limiting factor and time for microbial acclimation to the substrate. Isci and Demirer (2007) also reported a lag phase of 5-10 days in BMP assays involving cotton stalks, as the inoculum was not acclimated to the substrate. The cumulative biomethane volume at the end of the BMP assays increased with an increase in the ISR.

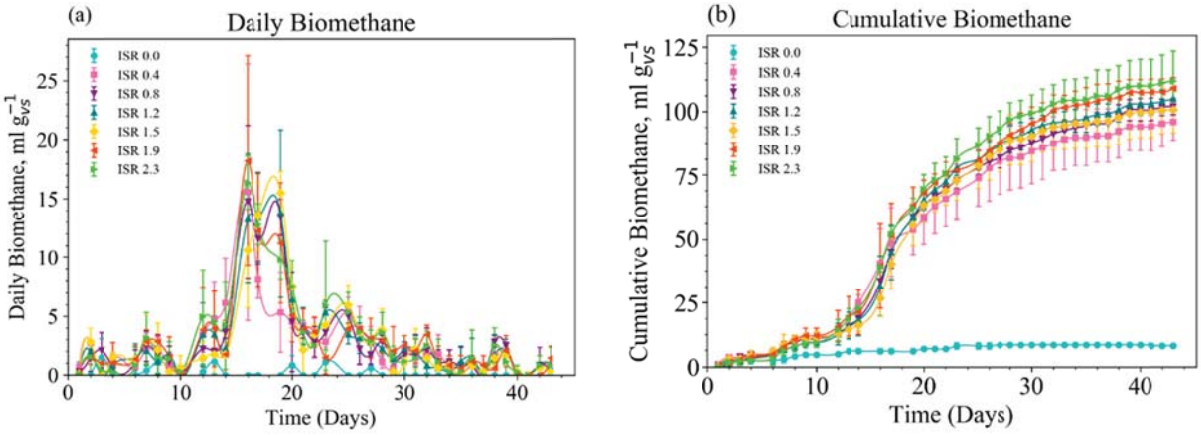


Figure 4. Daily (a) and cumulative (b) biomethane yields (mL g_{vs}^{-1}) from cotton gin trash at different inoculum to substrate ratios (ISRs)

Note. The error bars represent the standard deviation. Each dot on the chart represents the mean of three values.

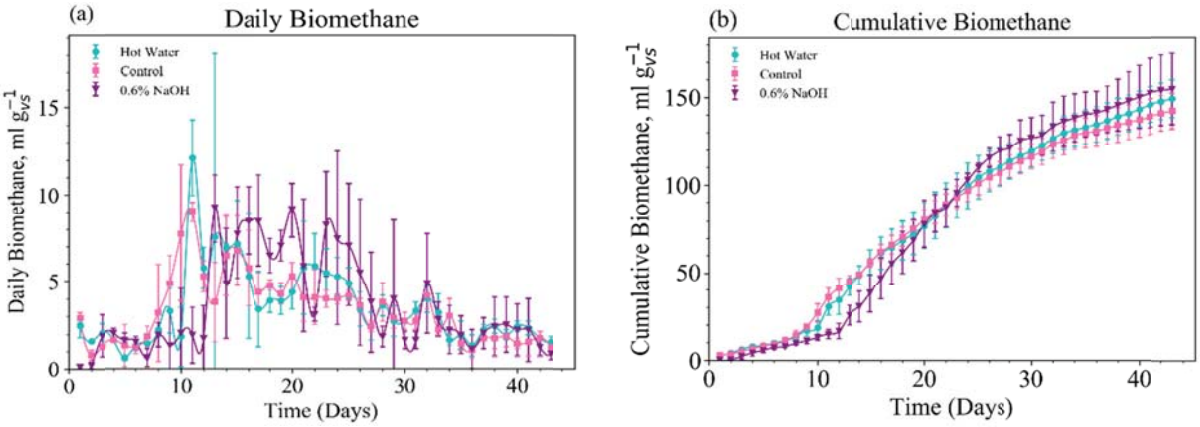


Figure 5. Daily (a) and cumulative (b) biomethane (mL g_{vs}^{-1}). from control (CGT), hot water, and 6% NaOH pretreatments

Note. The error bars represent the standard deviation. Each dot on the chart represents the mean of three values.

The biomethane recovered at the ISRs of 0.8.1.2, 1.5, 1.9, and 2.3 was similar ($P < 0.0001$). The cumulative biomethane measured from the control bioreactors was significantly ($P < 0.0001$) lower than all other treatments. At 0.4 ISR, the recovered biomethane was significantly ($P < 0.0001$) lower than all other ISRs (except 0 ISR). The low ISR may have caused biomethane inhibition due to the accumulation of volatile fatty acids (Angelidaki & Sanders, 2004). The technical digestion time ($T_{80\text{-}90}$) for biomethane production (the time taken to produce 80-90% of the cumulative biomethane) for the ISRs of 1.2 1.5 and 2.3 was found to be 26-30, whereas the $T_{80\text{-}90}$ for the ISRs of 0.4, 0.8 and 1.9 varied between 26-31, 27-32, and 27-31 days. The control yielded 80-90% of the biomethane between 20-23 days. Kafle et al. (2013) stated that $T_{80\text{-}90}$ could be used as hydraulic retention time (HRT) for the continuous anaerobic digestion of substrates. Adl et al. (2012) reported that the biomethane recovery from cotton stalks increased with an increase in ISR. Cheng and Zhong (2014) also reported that the biomethane yield from cotton stalks increased with an increase in ISR. They reported a biomethane yield of 113-180 mL g_{vs}^{-1} for the F/I (feed to inoculum ratio, VS basis) of 6-2. We observed cumulative biomethane in the range of 95.8-111.8 mL g_{vs}^{-1} for 0-2.3 ISRs (Table 2). Isci and Demirer (2007) reported a biomethane yield of 61.5 mL g_{vs}^{-1} for cotton stalks. The effect of ISR on the BMP of CGT has not been reported so far. Our findings corroborate with the observations by Ohemeng-Ntiamoah & Datta (2019) that ISR in BMP assays should be ≤ 2, but ≤ 1 should be avoided.

3.4 Biomethane Production Kinetics

The principal kinetic parameters of biomethane recovery from CGT during the anaerobic digestion were determined by employing the modified Gompertz model (Figures 6 and 7, Table 2). The model is most suitable for the growth curves with a prolonged lag phase as observed by the experimental daily biomethane yields (Figure 1). The simulated maximum biomethane yield was similar ($P < 0.0001$) to the experimental cumulative yields in both experiments. The maximum daily rate of biomethane production (R_m) was significantly ($P < 0.0001$) lower in ISR 0.4 than all other ISRs. The R_m for all other ISRs was similar. The λ among ISRs varied between 8.2±0.5 and 10.3±0.7 d. In this study, the inoculum was collected from a municipal wastewater treatment plant, while the substrate was a lignocellulosic agro-industrial waste. Hence, the high lag phase may be attributed to the microbial acclimation to the new substrate (Kythreotou et al., 2014).

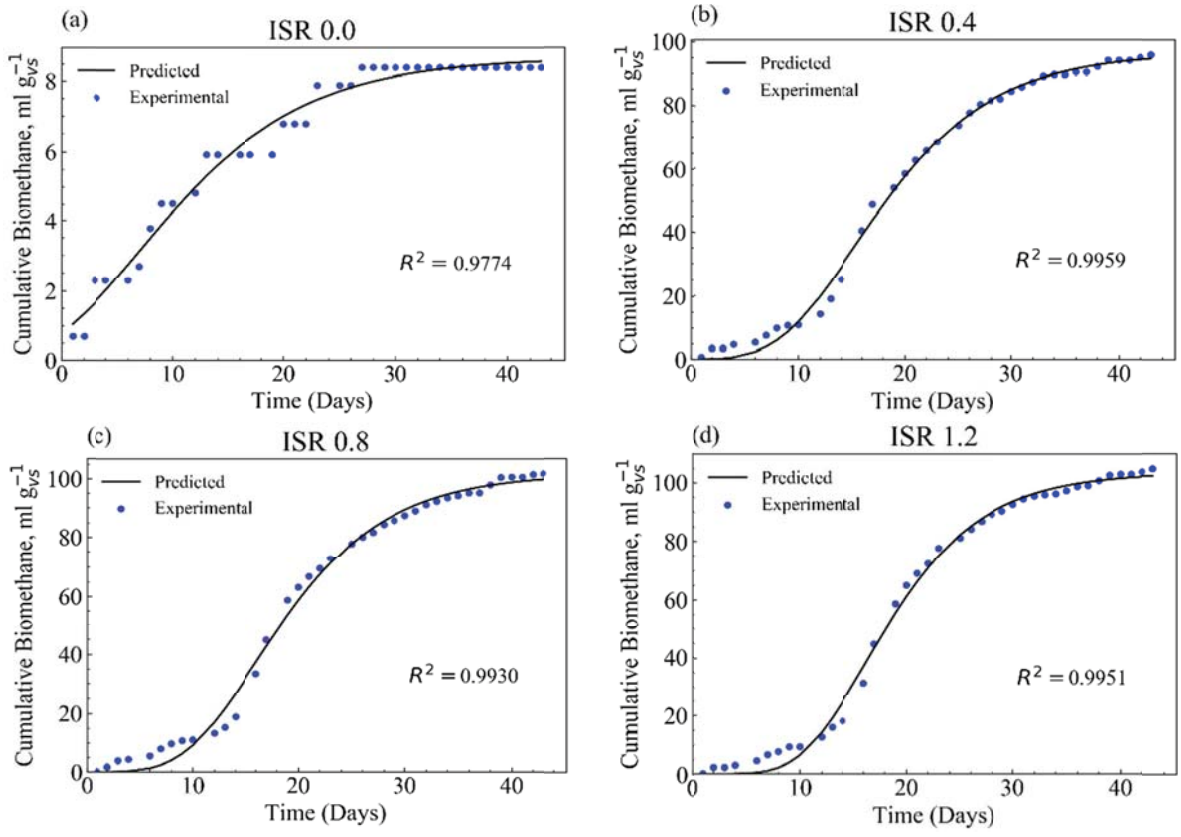
Among control, hot water, and alkali pretreatments, the prolonged lag phase of 9.8±1.0 d was observed in 6% NaOH, which corroborates with the observed daily values. In control and hot water treated CGT, the lag phase was 5.1±1.0 and 6.0±0.7, respectively. The high correlation coefficient (0.97-0.99) further supported that the equation is well fitted to the anaerobic digestion of CGT at all ISRs except control (0 ISR) and pretreatments. Cheng and Zhong (2014) also reported that the modified Gompertz equation fitted well to the anaerobic degradation of cotton stalks.

Table 2. Gompertz parameters, experimental biomethane, and biodegradability (BD) at different inoculum to substrate ratios (ISRs) and pretreatments

Treatment	P[1]	E[2]	R_m[3]	λ[4]	R^2	BD (%)
ISR Effect						
0 ISR	8.7±0.3 c	8.4±0.3 c	0.4±0.1 c	-1.1±1.6 c	0.977±0 b	1.8±0.1 c
0.4 ISR	96.6±6.6 b	95.8±7.2 b	5.0±1.2 b	8.2±0.5 b	0.996±0 a	21.2±1.6 b
0.8 ISR	101.8±4.6 ab	101.8±3.2 ab	5.5±0.6 ab	9.2±0.8 ab	0.993±0 a	22.6±0.7 ab
1.2 ISR	103.3±4.9 ab	104.7±4.6 ab	6.3±0.2 a	10.2±1.3 ab	0.995±0 a	23.2±1.0 ab
1.5 ISR	101.4±9.8 ab	100.5±9.3 ab	6.1±0.7 ab	10.3±0.7 a	0.992±0 a	22.2±2.1 ab
1.9 ISR	109.7±7.5 a	108.9±4.2 a	5.7±0.4 ab	8.8±1.9 ab	0.995±0 a	24.1±0.9 a
2.3 ISR	111.6±10.6 a	111.8±11.7 a	6.6±0.3 a	9.7±0.5 ab	0.994±0 a	24.8±2.6 a
LSD[5]	12.3	12.0	1.2	2.1	0.01	2.8
Pretreatment Effect						
Control	148.6±9.6 a	141.8±10.3 a	5.5±0.6 b	5.1±1.0 b	0.997±0 a	31.4±0.1 a
Hot water	157.5±3.9 a	148.9±10.8 a	5.7±0.8 b	6.0±0.7 b	0.997±0 a	31.7±2.3 a
6% NaOH	159.6±26.5 a	154.5±20.4 a	7.6±0.4 a	9.8±1.0 a	0.996±0 a	33.0±2.4 a
LSD	42.0	38.2	1.6	1.6	0.006	5.1

Note. [1] & [2] Simulated and experimental biomethane potentials (mL g_{vs}^{-1}), respectively; [3] Maximum daily rate of biomethane production; [4] Lag phase (d); [5] least significant difference.

Each value is a mean±SD from triplicates. The values across a column sharing the same letter are similar at α = 0.05.



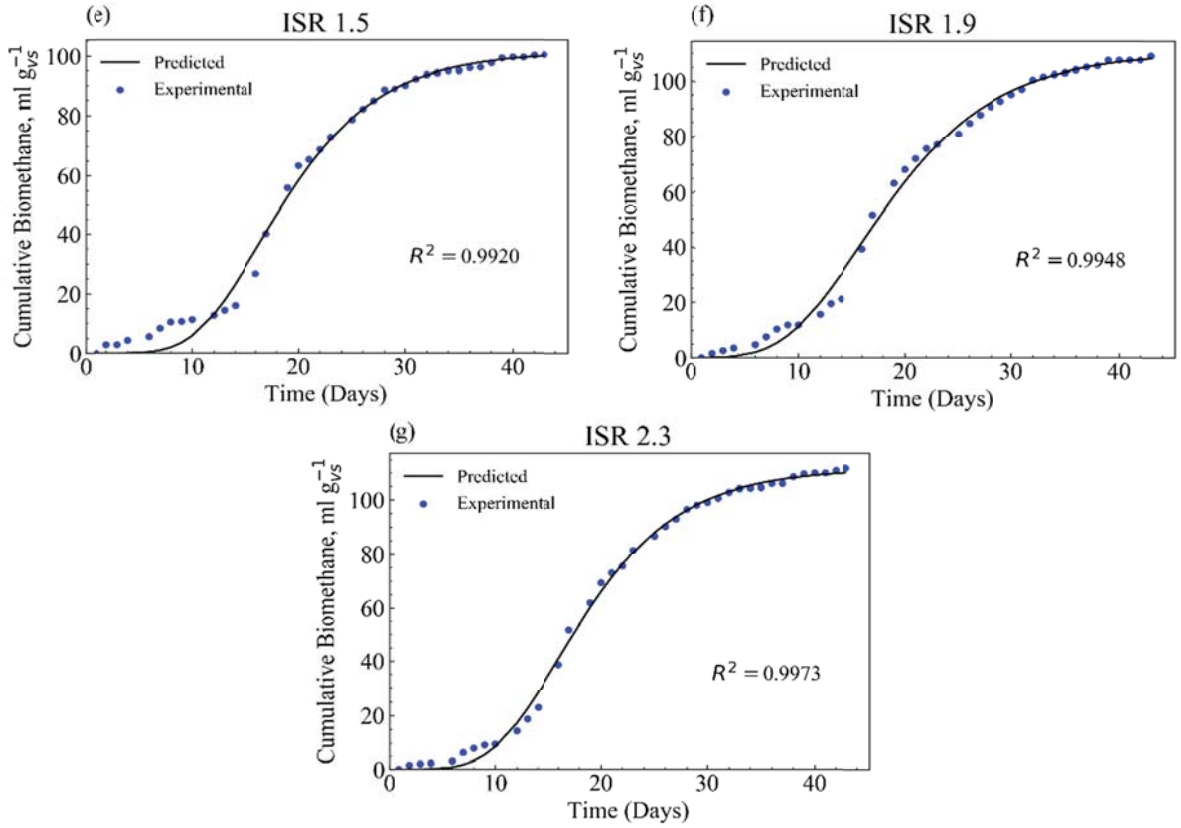


Figure 6. Experimental and predicted cumulative biomethane (mL g_{vs}^{-1}) at different ISRs

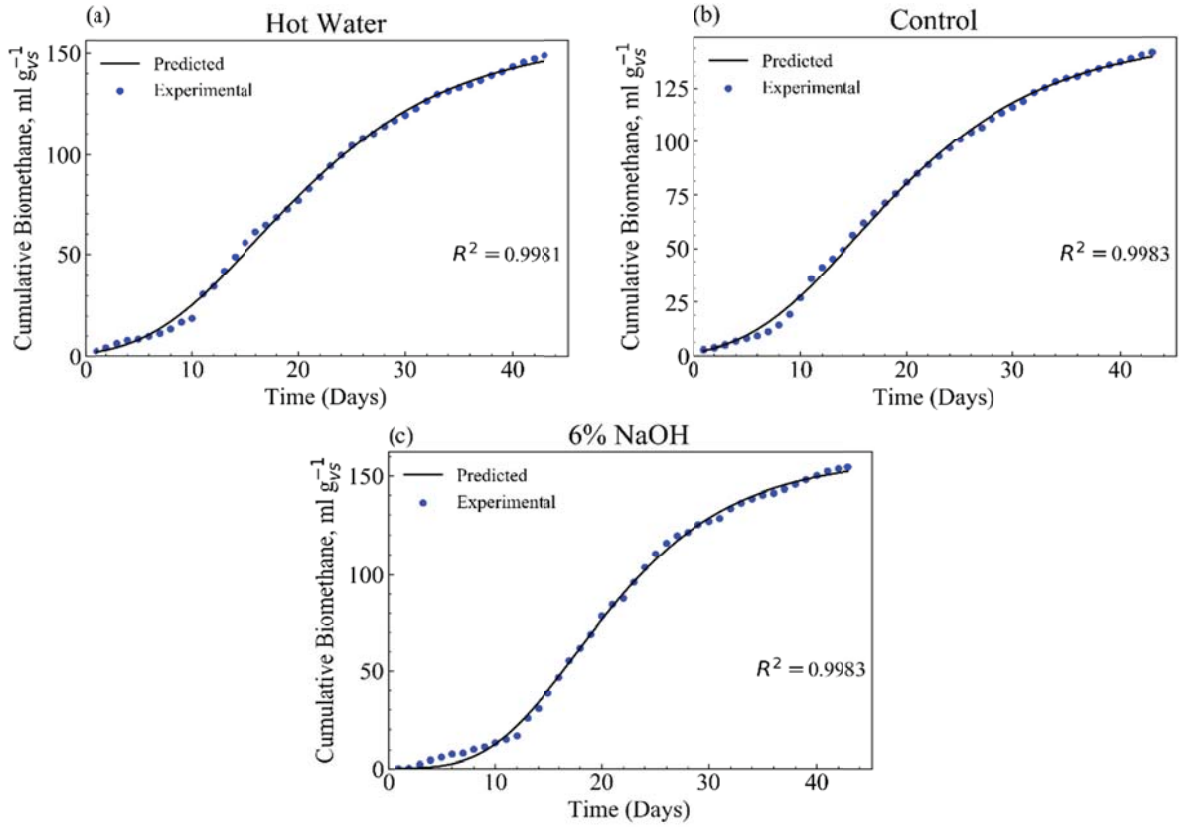


Figure 7. Experimental and predicted cumulative biomethane (mL g_{vs}^{-1}) in the hot water (a) control (b) and 6% NaOH pretreatments

3.5 Biodegradability

Without the addition of inoculum, CGT is hardly degradable, and only 1.8±0.1% of the added biomass was recovered as biomethane (Table 2).

In the pretreatment experiment, the BDs of 33.0±2.4, 31.7±2.3, and 31.4±0.1 were statistically similar in 6% alkali, the hot water treatments, and control, respectively.

Lignin is one of the most recalcitrant components of the plant-based agro-wastes and is not easily degraded during anaerobic digestion (Li et al., 2018). The lignin content of organic manures and energy crops, and animal manures is negatively correlated to their BMP (Kafle & Chen, 2016; Triolo et al., 2011). The CGT in this study had a lignin content of 32.7%, which on solubilization by alkali might have lead to higher BD (although not significant) in 6% NaOH treatment (Hassan et al., 2014). Shi et al. (2009) reported that a combination of fungal and alkali pretreatments in cotton stalks yielded higher biomethane by removing/softening recalcitrant biomass ingredients eg, xylan.

4. Conclusion

Our study reveals that for proper digestion of CGT the ISR should be more than 0.4. The pretreatments do not enhance biomethane yield and the modified Gompertz equation fits well with the anaerobic digestion of CGT. The gin trash biodegrades better with an increase in volatile solid loading rate from 1 to 7.9%. The $T_{80\text{-}90}$ for CGT at the ISRs of 1.2, 1.5, and 2.3 was 26-30 days, whereas $T_{80\text{-}90}$ at ISRs of 0.4, 0.8, and 1.9 were 26-31, 27-32, and 27-31, respectively. The 6% NaOH treatment significantly increased the biodegradability of cotton gin waste. These findings further enhance understanding of the underlying factors in the anaerobic digestion of CGT and will facilitate to maximize biomethane recovery from this agro-industrial waste.

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Evaluation of Methane Production From Dairy Cow Manure and Vegetable Waste

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Abstract

Dairy cow manure has high buffering capacity hence a substrate for anaerobic digestion, however the process is not optimised in mono-digestion system due to limited substrate. The aim of the study was to assess the effect of co-digesting animal waste and vegetable waste on methane production. Two systems were applied- batch and continuous anaerobic digestion system to determine effect on methane yield. The experiments were conducted with treatments as: manure alone (M), composite of manure with cabbage (MC), manure with potatoes (MP), manure with cabbage and potatoes (MCP), faecal alone (F), faecal with cabbage (FC), faecal with potatoes (FP) and faecal with cabbage and potatoes (FCP). Rectal grab samples were collected prior to incubation and manure was collected from the pens. All treatments were in replicates. Composite of manure or faecal with cabbage and potatoes produced the highest biogas (FCP: 32.1 mL/g DM, MCP: 29.5 mL/g DM) and methane (FCP: 3.13 mL/g DM, MCP: 2.36 mL/g DM) compared to manure alone or faecal alone (F: 27.0 biogas mL/g DM, M: 26.6 biogas mL/g DM) (F: 1.36 methane mL/g DM, M: 1.18 methane mL/g DM). Co-digesting dairy excreta with cabbage as only vegetable substrate affected anaerobic digestion (FC: 24.8 mL/g DM, MC: 24.9 mL/g DM), since it was the lowest in biogas production compared to all treatments. The anaerobic digestion system had an effect in methane production since continuous anaerobic digestion system produced the highest methane compared to batch anaerobic digestion system in all treatments. The results obtained in this study suggest that composite of manure with both cabbage and potatoes results in the highest biogas and methane production.

Keywords: biogas production, dairy cow manure, methane production, vegetable waste

1. Introduction

The Environmental consequences of animal manure disposal have motivated experts to seek for strategies which could lead to sustainable farming. Solutions that could help to transmute manure into value-added marketable products and environmentally friendly. Manure is one of the biodegradable products and should not be disposed in landfills since it contains substantial levels of harzadous pathogens and nutrients (Neshat et al., 2017). Any mismanagement of this valuable waste may lead to contamination of water sources, soil, air pollution, and harmful microbial build-up in the environment. Anaerobic digestion is one of the best waste management practices, whereby biogas and quality fertilisers are produced. Anaerobic digestion is the process by which microbes' breakdown organic material under oxygen free environment to produce mainly methane and carbon dioxide (Sebola, Tesfagioggis, & Muzenda, 2014).

An increase in human population has led to a mass-production of animals in farms to meet food requirement of the growing population. This in return results in high amount of manure produced by animals (Moeletsi & Tongwane, 2015). Management of huge amount of waste produced daily is a challenge and an understanding on how to wisely utilise manure on the farm is in demand. The nutrients in the manure makes it a valuable commodity, however the same nutrients in the manure makes it a threat to the environment (Mu et al., 2017).

Even though anaerobic digestion is the best alternative to manure disposal on open land, conversely, the low carbon to nitrogen ratio cannot successfully meet the requirement of anaerobic digestion (Neshat et al., 2017). Hence there is a need to co-digest manure with another carbon–rich substrate. Anaerobic co-digestion results in high production of biogas than mono-digestion. The characteristics of co-substrates utilised for co-digestion is vital; substrates with high carbon and low nitrogen are preferred. Vegetable waste such as cabbage and potatoes seem promising as they are rich in carbon and abundantly available at low cost throughout the year (Patil & Deshmukh, 2015). The current study was designed to assess the biogas production from dairy cow manure co-digested with vegetable waste and to assess the effect of batch anaerobic digestion and continuous anaerobic digestion on methane production. Dairy cow manure is one of the good substrates for anaerobic digestion because of its high buffering capacity, which aid in stable anaerobic digestion.

2. Methods

2.1 Study Area

The study was conducted at Agricultural Research Council (ARC) in Irene at Animal nutrition section, South Africa. The climatic classissification of the area is Cwb, which means subtropical highland climate.The area is situated in the Highveld at an altitude of 1525 m above sea level. The weather conditions range from hot days and cool nights in summer (17.5 °C to 32 °C) to moderate winter days with cold nights (1 °C to 17 °C) (Grobbelaar, Sutherland, & Molalakgotla, 2010).

2.2 Ethical Statement

The study was approved by Tshwane University of Technology ethics committee (Ref No: AREC2015/10/007) and Agricultural Research Council ethics committee (Ref No: APIEC16/027), before the experiments were conducted.

2.3 Substrates Collection

Fresh manure (2 Kg) was collected using a clean basket from the floor. Faecal samples (2 kg) were randomly collected using a clean and sanitized hand gloves. Faecal samples were collected from the dairy cows placed in a comfortable crush pen for easy rectum manure collection and hands were washed between each and every animal and gloves were changed between each animal. Collected samples were dried at 60 °C using an oven for 48 hours prior to grinding. The sample was grinded to 1mm particle size. The sample was stored in an air tight container away from the sun for further analysis. Vegetable wastes (cabbage and potatoes) were collected from Tshwane market and Marabastad and then washed (to remove soil particles). Cabbage and potatoes were dried at 60 °C for 24 hours in an air tight circulating oven. The sample was then grinded to 1mm particle size and stored in an air tight container.

2.4 Analytical Methods

All samples collected were dried and analysed in triplicates for DM, ash, EE and CP using laboratory standard procedures (AOAC, 2000). The CP was determined using the Kjeldahl method. The NDF, ADF and ADL were analysed following Van Soest et al. (1991). The pH was determined using extech 407228 pH/mV/Temperature Meter Kit (USA). The gas production was determined using ANKOM gas production system (ANKOM® Technologies corp., Fairport, NY) and methane content was determine using laser methane mini (LMm) ATEX-rated, laser-based remote methane. The biological characteristics of feedstuff used for batch and continuous digestion are shown in Table 1.

Table 1. Biological characteristics of substrates in Dry-Matter basis

Parameters	Potato wastes	Cabbage wastes	Manure	Faecal
% NDF	16.34	28.20	67.18	67.58
% ADF	3.76	7.88	30.25	27.25
% ADL	0.71	1.37	6.53	6.49
% CP	11.89	9.01	8.93	8.89
% EE	0.43	2.48	0.93	0.91
% DM	16.85	12.21	22.04	21.68
% CA	7.11	19.75	9.04	8.96
% HC	3.06	6.62	23.72	20.76
pH	5.0	6.63	6.61	6.65
% ODM	92.89	80.25	90.96	91.04

Note. % NDF: percentage Neutral Detergent Fibre, % ADF: percentage Acid Detergent Fibre, % ADL: percentage Acid Detergent Lignin, % CP: percentage Crude Protein, % EE: percentage Ether Extract, % DM: percentage Dry Matter, % CA: percentage Crude Ash, % HC: percentage Hemicellulose, % ODM: percentage Organic Dry Matter.

2.5 In vitro Gas Production Test

The bottle of 250 ml volume was used. The content was in the ratio of 1:1 (sample: water) and were manually stirred and incubated at 39 °C. The gas reading for each incubation bottle was recorded every hour using an Ankom gas module (Ankom Technology, Macedon, NY, USA).

ANKOM gas production system records gas production in pressure (psi). The continuous readings provided with automatic gas production make it possible to mathematically describe gas production curves (Cornou *et al*., 2013). The values recorded were converted to moles of gas produced using 'ideal' gas law and converted to millilitres of gas produced using Avogadro's law as stated by the ANKOM system.

The following equation was applied:

$$n = p \cdot \frac{V}{RT} \quad (1)$$

where, n = gas produced in moles (mol); p = pressure in kiloPascal (kPa); V = head space volume in the glass bottle in litres (L); T = temperature in Kelvin (K); R = gas constant (8.314472 L kPa K^{-1} mol^{-1}).

To convert gas volumes in mL at standard temperature and pressure, the Avogadro's law was applied. The law hypothesise that at atmospheric pressure measured in psi (1 psi = 6.8947577293 kiloPascal), 1 mole occupies 22.4 l at 273.15 °K and 101.325 kPa. The gas measured in moles was converted to gas measured in mL as follows:

$$\text{Gas produced in ml} = n \times 22.4 \times 1000 \quad (2)$$

2.6 Methane Anaerobic Digestion Set-Up

The batch and continuous fermentation system were set up. They were managed under anaerobic mesophilic condition (38-39 °C) with treatments: (A) control-manure/faecal, (B) manure/faecal and cabbage, (C) manure/faecal and Irish potatoes and (D) manure/faecal, cabbage and potatoes. In both fermentation systems, water to substrate ratio was 1:1 in 250 mL bottle and fermented for 120 hours. The continuous fermentation system was fed daily with the orgainic loading rate of 1 g/DM and the equal amount was discharged daily. The Methane was measured every 24 hours using laser methane mini (LMm) ATEX-rated, laser-based remote methane detector. The experiment was conducted as a Randomised Complete Block Design (RCBD) with different sample collection as a blocking factor. The experimental designs are shown in Table 2.

Table 2. Experimental design of manure co-digested with vegetable wastes

Sample	Treatments	Description
Manure from the floor	Manure (control)	100% manure
	Manure and cabbage	75% manure and 25% cabbage
	Manure and potato	75% manure and 25% potato
	Manure,cabbage and potato	75% manure, 12.5% potato and 12.5% cabbage
Faecal from the rectum	Faecal (control)	100% manure
	Faecal and cabbage	75% manure and 25% cabbage
	Faecal and potato	75% manure and 25% potato
	Faecal, cabbage and potato	75% manure, 12.5% potato and 12.5% cabbage

2.7 Characteristics Statistical Analysis

All the data such as potential gas production, rate of gas production, methane production and pH were subjected to analysis of variance using General Linear Model (GLM) procedures in Minitab Statistical Software, Version 17 (Minitab, 2010). Treatment means were compared using a Fishers' least significant difference (LSD) and significant differences were declared at $p < 0.05$.

3. Results

3.1 Effect of Dairy Manure Co-digested with Vegetable Waste on Biogas Production

Biogas production and rate of biogas production are shown in Table 3. The highest biogas production was achieved from co-digestion of dairy manure, cabbage and potatoes (FP: 32.6 mL, FCP: 32.1 mL, MCP: 29.5 mL). The lowest biogas production was observed from dairy manure co-digested with cabbage alone (MC: 24.9 mL, FC: 24.8 mL). The trend observed in all treatments was the same, whereby biogas production increased with time. However the rate of biogas production varied between treatments.

Table 3. Biogas production and rate of biogas production (mL/g DM)

Treatment	Parameter	
	Potential gas production	Rate of gas production
M	26.6±0.23 ^{b}	0.05±0.00 ^{b}
MC	24.9±0.59 ^{c}	0.06±0.04 ^{a}
MP	26.3±0.16 ^{b}	0.06±0.02 ^{a}
MCP	29.5±0.73 ^{a}	0.05±0.01 ^{b}
Average	26.83	0.05
F	27.0±0.13 ^{b}	0.05±0.00 ^{c}
FC	24.8±0.03 ^{c}	0.07±0.00 ^{a}
FP	32.6±0.04 ^{a}	0.06±0.00 ^{b}
FCP	32.1±0.32 ^{a}	0.07±0.00 ^{a}
Average	29.13	0.06

Note. ^{abc} Means within a column with different superscripts differ ($p < 0.05$).

3.2 Effect of Dairy Manure Co-digested with Vegetable Waste on Methane Production

Methane production from manure and faecal (rectal manure) co-digested with vegetable waste are illustrated in Figure 1. An increase in methane production was observed in all treatments co-digested with cabbage and potato waste. Anaerobic co-digestion of both cabbage and potato waste simultaneously attained the highest methane production (FPC: 3.13 mL, MPC: 2.36 mL). Co-digestion of dairy manure with either cabbage or potato waste produced more methane in comparison with the mono anaerobic digestion of manure or faecal sample (rectal manure). All treatments were observed to be significantly different ($p < 0.05$).

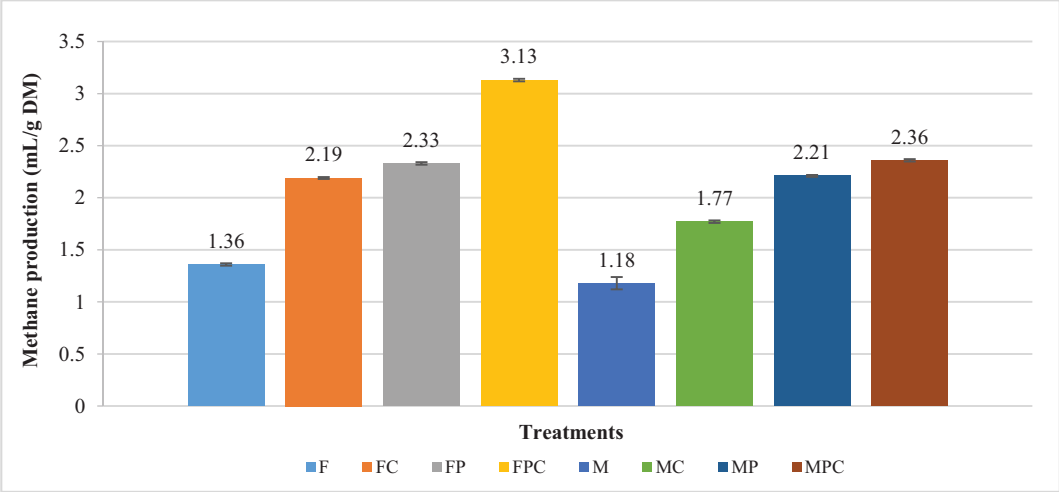

Figure 1. Methane production from manure/faecal co-digested with cabbage and potato waste

3.5 Batch and Continuous System Performance on Methane Production and pH

The performance of batch and continuous system on methane yield are shown in Figure 2. The methane production in the continuous system was high in all treatments compared to the batch system. Anaerobic co-digestion of manure with both cabbage and potatoes produced more methane in the batch system and in the continuous system. In continuous system, mono-anaerobic digestion of manure performed better in terms of methane production compered to anaerobic co-digestion of manure and cabbage.

The pH of the batch and continuous system are illustrated in Table 7. The initial pH of the batch and continuous system were the same, however the final pH between batch and continuous system was highly significant ($P < 0.05$). In all treatments a low pH was observed in batch system compared to continuous system.

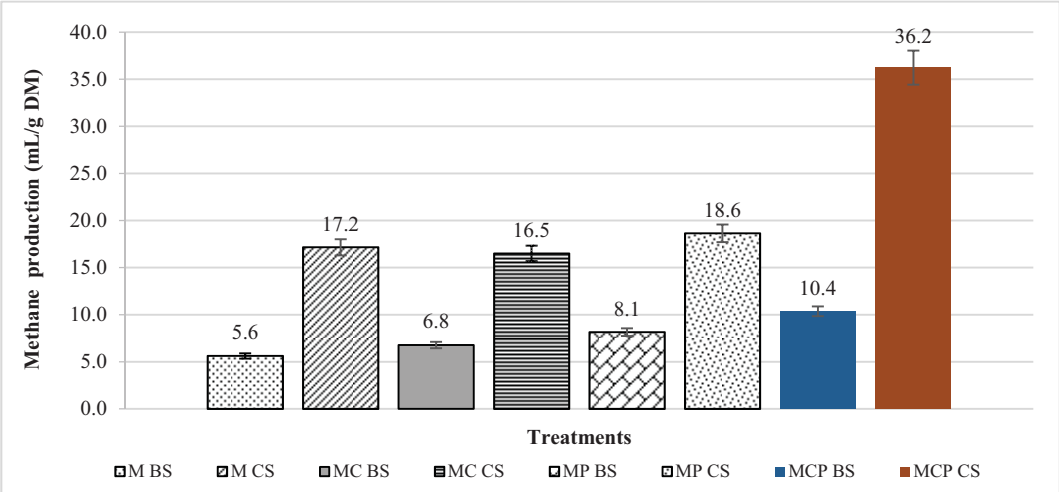

Figure 2. Performance of batch and continuous system on methane yield

Note. M BS: manure Batch system, MC BS: manure and cabbage wastes Batch system, MP BS: manure and potato wastes Batch system, MCP BS: manure, cabbage and potato wastes Batch system, M CS: manure continuous system, MC CS: manure and cabbage wastes continuous system, MP CS: manure and potato wastes continuous system, MCP: manure, cabbage and potato wastes continuous system.

Table 7. The pH of batch and continuous system

Treatments	Initial	Batch	Continuous
	0.0 hrs	120 hrs	120 hrs
M	6.8±0.38	6.2 ^{b}±0.47	6.4 ^{a}±0.49
MC	7.5±0.35	5.4 ^{b}±0.27	6.3 ^{a}±0.33
MP	7.1±0.20	6.1 ^{b}±0.31	6.9 ^{a}±0.44
MCP	7.4±0.19	6.3 ^{b}±0.63	6.8 ^{a}±0.11

Note. ^{ab} Means across a row with different superscripts differ ($P < 0.05$).

M: manure; MC: manure and cabbage wastes; MP: manure and potato wastes; MCP: manure, cabbage and potato wastes.

4. Discussion

4.1 Effect of Dairy Manure Co-digested With Vegetable Waste on Biogas Production

Co-digestion of dairy manure with vegetable waste enhances biogas production. The fermentation of single organic waste is more likely to fail and this may be due to free ammonia inhibition that exist when livestock manure are digested alone (Shah et al., 2015). The process of co-digesting more than one substrates improves nutrient balance, biogas production, control C/N ratio and reduces toxic compounds on the digestion process (Ajay et al., 2011). This statement is also supported by the study done by Mu et al. (2017) whereby the mixture of both cabbage and potatoes produced the highest biogas compared to single digestion of each vegetable waste. Co-digesting manure with vegetable wastes was evaluated in this study. It was observed that manure or faecal co-digested with both cabbage and potato wastes resulted in an increase of biogas production, compared to manure alone. Comparable results were obtained from the study done by Kassuwi, Mshandete, and Kivaisi (2012); however, manure was co-digested with cabbage only or potatoes only

The results from faecal (faecal collected from the rectum) and faecal co-digested with vegetable waste performed better in terms of biogas production compared to manure (manure collected from the floor) digested as a single substrate and manure co-digested with vegetable waste. This could have been initiated by microbial activities which were alive and already fermenting in the faeces, unlike in manure which was already exposed to aerobic conditions and uncontrolled temperature. From this study it was observed that biogas production from manure or faecal sample co-digested with cabbage only was low in biogas production compared to manure co-digested with potato waste. These results concurred with findings of Mu et al. (2017). The plausible reason is that potatoes are rich in starch, which is easily digested and contain 84% water, which enhance moisture content in the digester. Some studies have shown that biogas production and performance of the processes are significantly improved when manure is co-digested with both cabbage and potatoes at the ratio of 1:1. This balance the C/N ratio (2-30) which is desirable for biogas production (Kim et al., 2006; Liang & McDonald, 2015).

4.2 Effect of Dairy Manure Co-digested With Vegetable Waste on Methane Production

Biogas contains mainly methane that is essential for renewable energy. Various studies reported that co-digestion of manure with other organic substrates increased methane production and this could be attributed to additional nutrients such as carbon, phosphorus and nitrogen (Yadanaparthi & Chen, 2013; Mamun & Torii, 2015; Liang, Armando, & McDonald, 2015). In the current study, it was observed that the highest methane yield was from faecal or manure co-digested with both cabbage and potatoes. Potato waste as a co-substrate yielded higher methane compared to cabbage waste. The results are in agreement to those reported by EI-Mashad and Zhang (2010). Furthermore, similar results were observed from the study done by Yadanaparthi and Chen (2013) on co-digestion of manure with cabbage and potatoes using batch system. The plausible reason could be that the digestion time for cabbage takes longer compared to potatoes, moreover, the cabbage is high in nitrogen, which need more time to undergo hydrolysis and acidogenesis processes (Mu et al., 2017). On the contrary, Dandikas et al. (2014) observed the highest methane production from cabbage waste. The variation in the results reported by other authors and of this study could be due to pre-treatment of substrates before digestion and hydraulic retention time (Mu et al., 2017).

4.5 Batch and Continuous System Performance on Methane Production and pH

The pH in a bio-digester is a delicate parameter used to determine bio-digester stability. The optimum pH reported by several studies ranged between 6.1-8.3 (Yadvika et al., 2004; Kafle et al., 2013). Several studies documented that the pH below 6.1 and above 8.3 had a low methane production, since methanogens responsible

for methane yield are active when the pH is between 6.1- 8.3 (Kafle et al., 2013). In the current study, the pH in batch system was low compared to continuous system at the end of the digestion. However, all treatments in both systems were still at an optimum pH range. Sibiya, Muzenda, and Tesfagiorgis (2014) reported that the pH of the digester is mainly, affected by digester system. The continuous digester system is more stable compared to batch system. These results are in agreement with the results obtained by Deressa et al. (2015).

5. Conclusion

Based on the results, vegetable waste such as cabbage and potatoes are good substrates to co-digest simultaneously with dairy manure or faecal to enhance methane production. Cabbage wastes alone as a co-substrate for manure resulted in low pH and low methane production compared to potatoes waste and this is due to its rapid acidification. The batch digestion system can be used when substrates to be digested are limited. Continuous system was observed to double the amount of methane production compared to batch digester system and it can be used when substrates to be digested are not limited or are readily available.

Significant research efforts are needed regarding inhibitory factors during anaerobic digestion, optimum mixing ratio for different feedstuff and energy potential. Better operation techniques such as two-stage digesters and continuous digesters need to be investigated as well as appropriate organic loading rate and retention time of digestibility. This approach could enhance microbial hydrolysis of substrates and digestibility. In addition, more studies are required to understand types of pre-treatment methods that may be used to enhance digestibility of substrates during digestion.

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Yield and Yield Components of Potato (*Solanum tuberosum*) as Affected by Rock Phosphate in Standoff Soil, Southern Alberta Canada

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Abstract

An experiment was conducted in Standoff, Southern Alberta in April, 2020. The object of the experiment was to investigate effect of rock phosphate organic fertilizer on growth and yield of potato crop grown in Standoff. The varying levels of rock phosphate were broadcasted into the soil at control (0 P Kg ha^{-1}), Low P level (50 P Kg ha^{-1}) and High P level (100 P Kg ha^{-1}). The basal application of urea inform of nitrogen fertilizer was applied at 280 N Kg ha^{-1}. Potato seeds were planted at a distance of 30 by 90 cm. The three treatments were replicated three times, resulting into nine plants. One plant was taken out of uniformly grown tallest plant in each of the treatment to measure yield parameters. The yield parameters collected were subjected to analysis of variance (ANOVA) using Duncan's Multiple Range Test (DMRT) for separation of means. Results of the experiment indicated that High P and Low P rock phosphate fertilizer levels positively influenced weight of potatoes at 76 and 112 Days after sowing (DAS), respectively while High P rock phosphate fertilizer level got highest number of potatoes than Low P and control at 76 DAS. Furthermore, High P rock phosphate fertilizer level and control plots supported marketable number of potatoes at 76 DAS while High P rock phosphate fertilizer level favoured unmarketable number of potatoes at 112 DAS. It was quite obvious from the results that marketable weight of potatoes was positively influenced by High P rock phosphate level and Low P rock phosphate level at 76 and 112 DAS, respectively whereas unmarketable weight of potatoes was affected by High P rock phosphate fertilizer level at 112 DAS. These results revealed the beneficial use of rock phosphate for potato crop production

Keywords: rock phosphate, marketable weight, unmarketable weight, marketable number, unmarketable number, standoff, soil, potatoes yield

1. Introduction

Potato (*Solanum tuberosum*) is a staple food crop for First nations, Kainai Blood Tribe in Southern, Alberta and Canada as a whole. It is a tuber vegetable crop, which can be boiled or fried and eat with leafy vegetable. It could also be processed by food industries as a snack. Potatoes accounted for 376,826,967 metric tonnes of world production (FAO, 2016). In Canada, it is accounted for 4,324,110 metric tonnes of production (FAO, 2016). The factors that support growth and yield of potatoes are fertile soils, water, nutrients especially nitrogen and phosphorus, light and temperature (Ensign, 1935; Mugo, et al., 2020). Potatoes production could be supported by adequate nutrient management (Koch, M. et al., 2020), but soil degradation caused essential nutrient to be deficient in western Canada soils (Oldeman, 1994; FAO, 1995; Lakshminarayan et al., 1996; UNEP, 2000). Southern Alberta soils have been affected by soil degradation, whereby most of the essential nutrients are deficient especially nitrogen, phosphorus and potassium. However, managing the soil phosphorus in this region is very important to increase production of potatoes.

Rock phosphate fertilizer is an organic fertilizer that increase phosphorus in soil deficient P (Chien & Menon, 1995; Rajan et al., 1996; Zapata, 2003). It is a resource cheap fertilizer that is mined from sedimentary rock (Chien, 1992; Chien & Friesen, 1992; Chien & Van Kauwenbergh, 1992). Application of rock phosphate increase other nutrients in the soil such as nitrogen, potassium, magnesium, sulphur, calcium and some micronutrients (Zapata, 2003). Furthermore, phosphorous in form of mono or di phosphate was released to the

weakly acidic soil so as to increase crop yield and yield components (Jensen, 2010). However, the dissolved P in the soil can be taken up effectively by crops within the soil pH of 5.5 to 6.5 (Black, 1968; FAO, 1984; Jensen, 2010). Rock phosphate which contain lime materials is able to reduce the alkaline nature of soils for effectively P uptake by crops (Black, 1968; FAO, 1984; Zapata, 2003). Moreover, it has been discovered by Zapata and Roy (2004) that rock phosphate has residual effect, it builds up P for next cropping season. Nevertheless, many crops have been identified to use P from rock phosphate effectively (Flach et al., 1987; Kamh et al., 1999; Hocking, 2001; Montenegro & Zapata, 2002; Chien, 2003). However, most farmers in North America are still using water soluble fertilizer such as single supper phosphate, triple supper phosphate on their farms, not recognizing agronomical benefits of rock phosphate fertilizer. Therefore, the objective of this research effort was to evaluate effect of rock phosphate on the yield and yield components of potato planted in Standoff soil.

2. Materials and Methods

2.1 Site Description

The experimental trial was conducted in Standoff, Southern Alberta community garden. Standoff is a first nations, Kainai Blood Tribe (KBT) reserve community. It is located on latitude 49^{o} North and longitude 113^{o} West. Its location is on Hwy 2, 43 km South West of Lethbridge. Average temperature from April, 2020 to September, 2020 ranged between minimum of 7.6 ^{o}C to maximum of 20.7 ^{o}C while total daily rainfall was 261 mm from April, 2020 to September, 2020 (Agricultural Moisture Situation Update, 2021). Standoff is characterised by windy, dry and warm temperature in summer with little rainfall. Irrigation water was used to support little rainfall in the experimental site. Standoff soil is a Brown Chernozemic soils that are found in the Southern part of Alberta.

2.2 Physico-chemical Soil Composition

Soil samples from 0 to 15 cm layer were taken for physico-chemical analysis (Table 1). Nitrate-Nitrogen was extracted in the soil using 0.01M calcium chloride and N was detected by colorimetry. The phosphorus was extracted using modified Kelowna method and read by auto flow colorimeter while potassium was extracted from the soil using 1 N neutral ammonium acetate and K was detected by flame photometry. Micro nutrients were extracted from the soil using DTPA and measured by atomic absorption spectrophotometer (AAS). The soil texture was measured by hydromentry in soil samples dispersed in a water solutions of sodium hexametaphosphate. The major soil nutrients Nitrogen (N) was deficient, Phosphorus (P) was optimum and Potassium was in excess. Moreover. Secondary nutrients such as Calcium (Ca) and Magnesium (Mg) were at optimum levels whereas Sulphur (S) was deficient. Micro nutrients such as Zinc (Zn), Boron (B), Copper (Cu) and Sodium (Na) were at low levels while soil Iron (Fe) and Manganese (Mn) were in excess. The pH of the soil was 7.6 (1:1 soil:water). Soil textural class was silty clay loam. Southern Alberta soil is classified as Brown Chernozemic.

Table 1. Physico-chemical properties of potatoes field soil trial

Properties	Soil
N (Kg ha^{-1})	40.35
P (Kg ha^{-1})	97.50
K (Kg ha^{-1})	2006.32
S (Kg ha^{-1})	15.70
Ca (ppm)	4449.00
Mg (ppm)	510
Zn (ppm)	1.80
B (ppm)	0.90
Cu (ppm)	1.30
Fe (ppm)	23.00
Mn (ppm)	11.30
Na (ppm)	23.10
OM (%)	5.40
pH	7.6
EC	0.60
Saturated Bases (%)	
Ca	77.10
K	8.00
Mg	14.60
Na	0.30
ECEC	28.80
K/Mg	0.55
Sand %	19.9
Silt %	42.1
Clay %	38.0
Textural class	Silty clay loam

2.3 Experimental Design

The total area used in this trial plot was 450 m × 300 m = 135,000 m^2 The fertilizer was applied on April 30, 2020 at the rate of 100 P Kg ha^{-1} (High level), rate of 50 P kg ha^{-1} (Low level) and no application of fertilizer as control. The rock phosphate fertilizer was broadcasted to entire field according to P levels mentioned above. The basal application of nitrogen inform of urea was broadcasted at 280 N Kg ha^{-1} to entire experimental plot. The sangre potatoes variety were planted on May 8, 2020 at a space of 30 by 90 cm. Sangre is a new potato variety, dark red-skinned, white-fleshed oval potato recommended for boiling. Sangre potato variety is a mid to late season maturing with excellent tuber set and good yields. The treatments (Low P, High P and Control) were replicated three times, resulting into nine plants. One plant was taken from uniformly grown tallest plants in each of the treatment. The plant taken in each of the treatment was used to measure agronomic parameters: number of potatoes was measured by counting, weight of potatoes was measured by sensitive electronic weighing scale (Sartorius Lab. Instruments, GMBH & Co, Germany-ENTRIS 2202-1SUS), marketable number of potatoes was measured by counting harvested number of potatoes that weighed more than 33 g in each replicate and unmarketable number of potatoes was measured by counting harvested number of potatoes that weighed less than 33 g in each replicate, marketable and unmarketable weight of potatoes were measured by weighing potatoes that weighed more than 33 g and less than 33 g, respectively and residual phosphorus level in the soil after harvest was measured by using modified Kelowna method and read by auto flow colorimetry. The agronomic parameters were collected from May 8, 2020 when potato seeds were planted to September 15, 2020 when matured potatoes were harvested, resulting to total experimental period of 131 days after sowing.

2.4 Statistical Analysis

The agronomic parameters measured were subjected to analysis of variance (ANOVA) using IBM SPSS version 27 software, Duncan's Multiple Range Test was used for separation of means.

3. Results

3.1 Effect of Rock Phosphate Fertilizer on Weight of Potatoes and Number of Potatoes

Table 2 shows effect of varying levels of rock phosphate fertilizer on weight of potatoes and number of potatoes. Weight of potatoes was significantly influenced by rock phosphate fertilizer. It was obvious from Table 2 that High P rock phosphate treated plot had higher potato weight (655.50 g) than either Low P rock phosphate treated plot or control at 76 DAS, whereas at 112 DAS, Low P rock phosphate treated plot gave higher weight of potatoes (2038.10 g) than either High P rock phosphate treated plot or control. There was a marked increase of 210.9% from 76 DAS to 112 DAS, when soil was treated with high and low rock phosphate fertilizer. There was no effect in the effort of the treatments to support weight of potatoes at 98 and 131 DAS. Furthermore, number of potatoes produced was significantly highest at 76 DAS, when high P rock phosphate treated plot produced highest number of potatoes (16.30) than Low P rock phosphate treated plot and control. Thereafter, there was no significant effect of the treatments to support number of potatoes.

Table 2. Effect of rock phosphate fertilizer on weight of potatoes and number of potatoes produced over time

Treatments	Days After Sowing							
	Potatoes Weight (g)				Number of Potatoes			
	76	98	112	131	76	98	112	131
Control	335.90b	252.02	268.90b	1243.10	9.30b	8.30	6.30	6.30
Low P	155.60b	1509.50	2038.10a	5497.70	6.00b	13.30	9.30	11.00
High P	655.50a	1610.40	1225.80b	2205.60	16.30a	10.70	10.30	12.00
SE	125.30	304.90	251.50	2439.20	3.70	4.50	2.50	3.50

Note. Means with different letters are significantly different according to Duncan Multiple Range Test (DMRT) $p < 0.05$.

SE: Standard Error.

3.2 Effect of Rock Phosphate Fertilizer on Marketable and Unmarketable Number of Potatoes

Table 3 shows effect of varying levels of application of rock phosphate fertilizer on marketable and unmarketable number of potatoes. It was clearly seen from Table 3 that marketable number of potatoes at 76 DAS in High P rock phosphate treated plot and control plot jointly produced higher marketable number of potatoes than Low P rock phosphate treated plot, whereas High P rock phosphate treated plot gave higher unmarketable number of potatoes than either Low P rock phosphate treated plot or control at 112 DAS.

Table 3. Effect of rock phosphate fertilizer on marketable and unmarketable number of potatoes

Treatments	Days After Sowing							
	Marketable Number				Unmarketable Number			
	76	98	112	131	76	98	112	131
Control	6.30ab	7.30	6.00	5.70	3.0	1.0	0.30b	0.70
Low P	2.30b	8.70	9.30	9.70	3.7	1.0	0.00b	1.30
High P	11.00a	9.70	8.00	7.30	5.3	4.7	2.30a	5.00
SE	1.90	3.50	2.30	2.0	2.0	2.0	0.39	2.20

Note. Means with different letters are significantly different according to Duncan Multiple Range Test (DMRT) $p < 0.05$.

SE: Standard Error.

3.3 Effect of Rock Phosphate Fertilizer on Marketable and Unmarketable Weight of Potatoes

Table 4 reveals that High P rock phosphate treatment significantly gave higher marketable weight of 585.30 g than either Low P rock phosphate treatment or control with marketable weight of 112 .60 g and 294.60 g for Low P rock phosphate treatment and control, respectively at 76 DAS. High P rock phosphate treatment gave marked increase of 98.70% over control at 76 DAS. Furthermore, Low P rock phosphate treatment produced higher marketable weight (2037.90 g) than either High P rock phosphate treatment or control plots with 1179.20 g and

965.5 g, respectively at 112 DAS. Low P rock phosphate treatment had an increase of 111.10% over control at 112 DAS. There was no significant effect in the effort of the treatments to support marketable weight of potatoes at 98 and 131 DAS. Moreover, unmarketable weight of potatoes was observed at 112 DAS only, where High P rock phosphate treatment gave higher weight of 44.50 g than either Low P treatment or control with unmarketable weight of 0 g for Low P rock phosphate treatment and 3.20 g for control.

Table 4. Effect of rock phosphate fertilizer on marketable and unmarketable weight of potatoes

Treatments	Days After Sowing							
	Marketable Weight (g)				Unmarketable Weight (g)			
	76	98	112	131	76	98	112	131
Control	294.60b	239.60	965.50b	1222.40	40.90	10.70	3.20b	20.70
Low P	122.60b	1412.20	2037.90a	2102.20	33.10	95.00	0.00b	28.60
High P	585.30a	1569.90	1179.20b	2106.20	62.80	38.60	44.50a	98.40
SE	106.13	287.60	248.00	498.20	26.40	36.00	10.40	53.50

Note. Means with different letters are significantly different according to Duncan Multiple Range Test (DMRT) $p < 0.05$.

SE: Standard Error.

3.4 Residual Phosphorus Level in the Soil After Potato Harvest

Table 5 shows residual phosphorus levels in the soil after potato harvest. There was no significant difference in residual P level in the treated soil with rock phosphate fertilizer and control.

Table 5. Residual Phosphorus level after potatoes harvest

Treatments	Residual Phosphorus Level (Kg/ha) 131 Days After Sowing
Control	38.50
Low P	46.30
High P	67.60
SE	23.20

Note. Means with different letters are significantly different Duncan Multiple Range Test (DMRT) $p < 0.05$.

SE: Standard Error.

4. Discussion

Potatoes gained weight at 76 DAS, when rock phosphate was applied at high rate of 100 P Kg ha^{-1}. It was also observed at 112 DAS that low rate of 50 P Kg ha^{-1} positively influenced weight of potatoes, thereafter, there was no significant effort of the applied rock phosphate fertilizer to support weight of potatoes. This outcome reveals that concentration of rock phosphate applied may not be enough to support yield of potato crop beyond 112 DAS. Incorporation of large applications of PR (500-1000 Kg ha^{-1}) followed by a regular maintenance application of P would increase availability of P in the soil, as well as maintain the P in the soil (Zapata & Roy, 2004). Furthermore, rainfall data collected in the experimental site revealed inconsistent of rainfall (261 mm) and shortage of irrigation water during hot Summer period which contributed to low solubility of rock phosphate to support effectiveness of phosphorus uptake by plant for increase in potato yield (Agricultural Moisture Situation Update, 2021). It was clearly seen in our results that rock phosphate has no effect at 98 DAS and 131 DAS due to inadequate of soil moisture to dissolve rock phosphate. It has been confirmed by Weil et al. (1994) that rainfall is the most important climate factor that influences PR dissolution and its agronomic effectiveness. It was also stated by Weil et al. (1994) that increased soil moisture brought about by rainfall or irrigation, increases PR dissolution. The highest number of potatoes were produced from the plot treated with high P (100 P Kg ha^{-1}) at 76 DAS which indicated that large application of rock phosphate above 100 P Kg ha^{-1} could influenced number of potatoes (Weil et al., 1994).

Our result observed that control experiment with no rock phosphate fertilizer application and high P rock phosphate treatment favoured marketable number, whereas high P rock phosphate treatment supported

unmarketable number. This signifies that rock phosphate applied was not enough to support marketable number of potatoes (Perrott et al., 1996; Rajan et al., 1996).

Our result also revealed that application of high P rock phosphate treatment gave highest potato marketable weight than other treatments at 76 DAS while low P rock phosphate treatment significantly favoured highest marketable weight of potatoes than other treatments including control at 112 DAS. Moreover, unmarketable weight of potatoes was positively influenced by high P rock phosphate treatment at 112 DAS. High and Low rock phosphate applied to the soil able to support growth and yield of potato crop at 76 and 112 DAS due to favourable growing condition. However, inconsistent of rainfall (261 mm) and shortage of irrigation water at 98 and 131 DAS negatively influenced dissolution of rock phosphate. This was also confirmed by Perrott et al., (1993); Perrott and Wise (2000) that application of P would sustain P availability in the soil, as well as availability of moisture to dissolved rock phosphate.

After potatoes were harvested on the field, residual level of P was noticed in the soil that was treated with high P rock phosphate plot followed by low P treated rock phosphate plots while control gave the least, but there was no significant difference in the treated soils with rock phosphate and control, which indicated that rock phosphate applied at varying levels were not enough (Hedley & Bolan, 1997; Sale et al., 1997). However, Alberta Agriculture Food and Rural Development (2005) stated that the values of residual phosphorus obtained in this present study (38.50 P Kg ha^{-1} for control experiment and 46.30 P Kg ha^{-1} for Low P rock phosphate treated soil) were marginal P level in Alberta soils while 67.60 P Kg ha^{-1} for High P rock phosphate treated soil was adequate P level in Alberta soils. This confirmed that there was considerable amount of residual P in the soil after potatoes were harvested.

5. Conclusion

Direct application of rock phosphate is beneficial for potato crop production in Kainai Blood Tribe Southern Alberta soil. The rock phosphate organic fertilizer influenced potatoes yield, but we discovered that application of rock phosphate rates applied at 50 and 100 P Kg ha^{-1} were not enough to give real potatoes yield for the present study, as well as insufficient of soil moisture inform of rain fed or irrigation to dissolve P in rock phosphate for effective P uptake by potato crop. However, there was considerable quantity of P left in the soil after harvest. The P left in the soil could be used by plants in the next growing season. I would recommend that this trial should be repeated with higher rate of P than rate of P used in this experiment. Irrigation facilities must also be installed to supply water to soil for dissolution of rock phosphate for easy P uptake by potato crop, if there is no natural rainfall.

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Influence of *Diceraeus melacanthus* (Hemiptera:Pentatomidae) on the Development and Reactive-Oxigen-Related Enzyme Activity of Corn Seedlings

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Abstract

The damage caused by insect pests in plants can considerably affect their development and crop yield. It can also cause the activation of biochemical defense mechanisms in the plant, combined with the production of reactive oxygen species. The levels of these oxygen molecules are controlled by antioxidant enzymes and this mechanism is an important plant defense process. The aim of this study is to learn the effect of feeding by the stink bug, *Diceraeus melacanthus,* on the growth of corn plants and on the activity of the antioxidant enzyme peroxidase (POD) and catalase (CAT). The study was carried out in a greenhouse, in a completely randomized design, with a 2 × 4 factorial block and five replications. The first factor was composed of plants with and without the presence of the bug and the second factor was composed the age of corn plants, two, four, six, and eight days after emergence. Corn seedlings infested with the stink bug were negatively influenced in terms of plant height, root mass and increasing in activity of the POD enzyme was 84% higher in plants infested at 8th DAE. The CAT enzyme activity was not detected in the samples. The stink bug feeding affected the growth of the corn seedlings. The analysis of the POD enzyme could be a valuable tool to quantify the stress caused by the stink bug in futures studies.

Keywords: green belly stink bug, corn pests, injuries, antioxidant enzymes, peroxidase and catalase

1. Introduction

The corn plant *Zea mays* L. (Poaceae) can be affected by several pests that attack from the root, to the stalk and leaves, to the corn cob, causing damage throughout the cultivation cycle, if not properly managed. Among the pests, the green belly stink bugs species *Diceraeus melacanthus* Dallas, 1851 and *Diceraeus furcatus* Fabricius, 1775 (Heteroptera: Pentatomidae) are causing significant damage to the crop (Fernandes et al., 2020).

The stink bugs of the genus *Diceraeus* spp. (Hemiptera: Pentatomidae) gained prominence in the main producing regions, during the planting period of the second harvest. The dynamics of cultivation, such as, the anticipation of the soybean harvest and the consequent planting of corn in mid-January and February, coincide with the peak period of the stink bugs, which may culminate in significant damage to the crops (Chocorosqui & Panizzi, 2004). The greater occurrence of this pest is also related to the systematic adoption of the succession of soybean crops by corn crops and to the 'no-tillage system', which promotes favorable conditions for the development of phytophagous insects. So far, there exist a few control strategies that work efficiently (Chiesa et al., 2016).

The species *D. melacanthus* has a predominant occurrence in Brazil. Its presence in crops is related to its more active behavior in hot regions, with subtropical and tropical climate conditions, where the main agricultural areas of corn cultivation in the country are located (Chocorosqui, 2001). This insect feeds on cellular compounds of the plant's parenchymal tissue and xylem vessels (Lucini & Panizzi, 2018), preferring the base of the corn seedling stem as the main feeding point (Panizzi & Lucini, 2019). The potential for injuries caused by this stink bug can be particularly observed from nymphs of the fourth and fifth instar to adults, developmental stages that are responsible for causing significant damage to corn (Fernandes et al., 2020).

The injuries caused by *D. melacanthus* can vary from small perforations in the leaves, the induction of the tillering of the plant, injuries and withering of the leaves, and even more severe damage such as destruction of the apical meristem that can kill the plant. The attack of this stink bug affects the development of the plant, and makes it less vigorous, resulting in a reduced plant height and number of developed leaves. This impact can compromise the root system and normal development of the plant, resulting in negative results in grain productivity (Roza-Gomez et al., 2011; Crosariol Netto et al., 2015; Bridi et al., 2016).

Besides affecting the development of the plant, the stink bug feeding can induce the activation of defense mechanisms (Bi & Felton, 1995). One of the mechanisms triggered by plants is the production of defensive biochemical compounds in response to the herbivorous activity. Among these compounds are the reactive oxygen species (ROS) (Inzé & Montagu, 1995; War et al., 2012).

The most common ROS in plants are hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and the superoxide anion (O_2^-). These molecules are accumulated in parts of plants during the period of stress suffered by the induction of some agent, however, they are toxic both to the stressor and to the plants themselves. Consequently, the plant uses mechanisms to produce a complex of antioxidant enzymes that are able to fight and decompose the formed ROS, so that the plant can return to its normal biochemical state. The main antioxidant enzymes produced by plants that aid in the degradation process of ROS are, superoxide dismutase, peroxidase, glutathione reductase, catalase, and polyphenol oxidase (Maffei et al., 2007; Soares & Machado, 2007; Nascimento & Barrigossi, 2014).

Hydrogen peroxide (H_2O_2) is one of the main ROS formed by the plant and is closely linked to the plant's defense process. It can be considered as a type of response to stress caused by insect pest damage (Soares & Machado, 2007). The degradation of this ROS is a major function of the enzymes catalase and peroxidase, so to study the enzyme activity is very interesting after insects feeding. It is suggested, then, that the expression of these specific enzymes is a part response and strategy of induced resistance caused by insect feeding (Nascimento & Barrigossi, 2014).

According to the content explained, the study aimed to learn the effect of the presence of *D. melacanthus* on the development of corn plants and on the activity of the antioxidant enzymes catalase and peroxidase.

2. Material and Methods

The experiment was carried out in a greenhouse and in the Entomology and Biochemistry Laboratories at the State University of Maringá (UEM), Umuarama Campus, PR, Brazil in November 2017.

First-generation adult stink bugs reared in the laboratory were used to carry out the tests. The breeding was initialized with adult stink bugs captured in the field on host plants and kept in the Entomology laboratory under controlled conditions of temperature (25±2 ° C), relative humidty (65±10%) and a photoperiod of 12 hours.

The insects were kept in transparent plastic boxes (40.8 cm × 29.0 cm × 12.6 cm) with perforations for gas exchange. The rearing box was lined with filter paper that remained moist throughout the period. Cotton was also deposited into the boxes to serve as oviposition sites for stink bugs, which were fed with a natural diet, composed of fresh bean pods (*Phaseolus vulgaris* L., Fabaceae), tree privet fruit (*Ligustrum lucidum* W. T. Aiton, Oleraceae.), dried soybeans (*Glycine max* L. Merril, Fabaceae) and corn seedlings (*Z. mays*), according to methodology adapted from Chocorosqui and Panizzi (2002).

The experiment was carried out under greenhouse conditions in a completely randomized design with eight treatments, divided into a 2 × 4 factorial scheme and five replications. The first factor consisted of plants with and without the presence of the bug. The second factor was represented by the age of the corn plants and the consequent period of coexistence between pests and plants, corresponding to 2, 4, 6, and 8 days after emergence (DAE).

The experimental unit consisted of a polyethylene bag with capacity of 5 dm^3 of soil, where just one corn plant of the conventional hybrid Pioneer® 30F53 was grown. One adult stink bug non-sexed with 15 old day was artificially infested in each plant, the infestation occurred immediately after the emergence of the corn seedlings.

All plants in the experimental units (including those that were not available for the consumption of *D. melacanthus*) were surrounded by cloth "voile", attached by rubber band around the polyethylene bag, forming a study arena, not possibility entry and exit of others insects. Before infestation, all stink bugs were kept without food for a period of 12 hours.

The agronomically relevant attributes, such as plant height, root length, dry mass of aerial part (DMAP), dry root weight (RDM), and the activity of antioxidant enzymes peroxidase (POD) and catalase (CAT) were evaluated on the respective days of development of plants (2, 4, 6, and 8 DAE), both in the treatments with and without presence of bugs, following were be evaluations of destruction in the plants. The datas were collected until 8th DAE, the most susceptible phase of the crop to attack by the stink bug.

To quantify the activity of CAT and POD enzymes, parts of the basal leaves and stems of the seedlings, about 0.5 g, were used, which were homogenized in a previously cooled mortar (4±2 ºC) with 5.0 ml of potassium phosphate buffer extraction medium [67 mM], pH = 7.0, and 0.1 g of 1% PVP (w/v) (polyvinylpyrrolidone). The homogenate was centrifuged at 3000 rpm for 10 minutes in room temperature and the supernatant was used as an enzyme extract.

The POD was quantified with an aliquot of 500 µl of the plant extract that was added to 3 ml of the reaction medium consisting of 25 mM potassium phosphate buffer, pH = 6.8, 50 µl of H_2O_2 (10 mM), and 100 µl of guaiacol (2.58 mM). The POD activity was read using spectrophotometer at a wavelength of 470 nm, and the increase in absorbance was measured using the extinction coefficient of 25.5 mM^{-1} cm^{-1} (Pütter, 1974). The result was expressed in nmol of tetraguaiacol produced min^{-1} mg protein $^{-1}$.

For CAT, a 100 µl aliquot of the plant extract was added to 3 ml of the reaction medium, consisting of 25 mM potassium phosphate buffer, pH = 7.0, and 50 µl of H_2O_2 (10 mM). The CAT activity was read using spectrophotometer at a wavelength of 290 nm, and measured by decrease of absorbance using the extinction coefficient of 0.0394 mM^{-1} cm^{-1} (Aebi, 1984). The result was expressed in nmol of H_2O_2 consumed at min^{-1} mg protein $^{-1}$.

The data obtained from plant height (HT), root length, DMAP, RDM, and POD enzyme activity were submitted for analysis to check the homogeneity of the variances by the Brow Forsythe test, and normality by the Kolmogorov-Smirnov test. Having met these requirements, the data were subjected to analysis of variance (ANOVA) using the F test and the means were compared using the Tukey test ($P \leq 0.05$), and when necessary, the data were transformed into Log (X). For statistical analysis, the SAS 9.4 software was used (Sas Institute, 2013).

3. Results

There was a significant difference in the plant age factor in all variables analyzed ($P \leq 0.05$). The influence of the insect factor, characterized by the presence or absence of the stink bug, *D. melacanthus*, was observed for plant height, RDM, and POD enzyme activity. In the interaction between the factors of plant age and insects, significant differences were observed only for the enzyme POD ($F = 8.94$; $P \leq 0.05$) (Table 1).

Table 1. Two-way ANOVA summary for the variables analyzed: plant height (HT), root length (RL), dry mass of aerial part of plants (DMAP), root dry mass (RDM), and peroxidase enzyme (POD)

Variation	Calculated *F* value				
	HT	RL	DMAP	[1]RDM	[1]POD
Age (A)	12.38*	7.07*	40.81*	56.07*	5.42*
Insect (I)	25.81*	3.11^{ns}	0.00^{ns}	5.43 *	60.02*
(A×I)	1.23^{ns}	0.49^{ns}	1.15^{ns}	2.00^{ns}	8.94*
C.V. (%)	14.33	27.02	22.33	14.59	45.95

Note. *, ^{ns}: Significant at 5% probability and not significant, respectively.

[1] Data transformed into Log X.

C.V. (%): Coefficient of variation.

The heights of the corn plants were influenced by the insect factor, due to the presence or absence of the stink bug *D. melacanthus,* and by the age of the plants (Table 2). A reduction in the growth of the plants was attributed to the presence of bugs compared to plants without bugs. Insect-infested plants showed decreased growth and

development from the second DAE, with an average reduction of 23.5%. This pattern of impaired plant growth was observed until the eighth day after emergence of corn seedlings, with an average reduction of 26.8%.

No significant difference was observed for root growth and DMAP in all evaluations. These parameters were similar affected in plants with and without stink bug infestation.

When the RDM was evaluated at 2nd DAE, it was observed that there was no significant difference in the root mass between the roots with the presence or absence of the pest. This short period of bug-plant coexistence was not enough for the manifestation of changes in plant structure, however, the root mass of the plants was negatively altered at 4 and 6 DAE. It was noted that with a longer period of coexistence of the pest and plant, there were significant losses of mass of the root system. In implying a decrease in the RDM. In relative terms, the decrease in root mass was 55% and 67%, at 4th and 6th DAE, respectively. However, on 8th DAE, there was observed a recovery of the root mass in contrast to the decrease in dimension and mass of aerial plant parts.

The presence of *D. melacanthus* resulted in a constant increase in POD activity according to the time after infestation. Differences in POD concentration there was not occurred 2th DAE, probably due to the short time of establishment of bugs in plant populations, implying little impact in this period, and consequently low plant response to the presence of the stink bug. On 8th DAE, the plants infested with the bug showed 84% higher POD activity, when compared to plants kept without the insect, inferring the oxidative stress state of the plants, in response to the injury caused by the pest (Table 2 and Figure1).

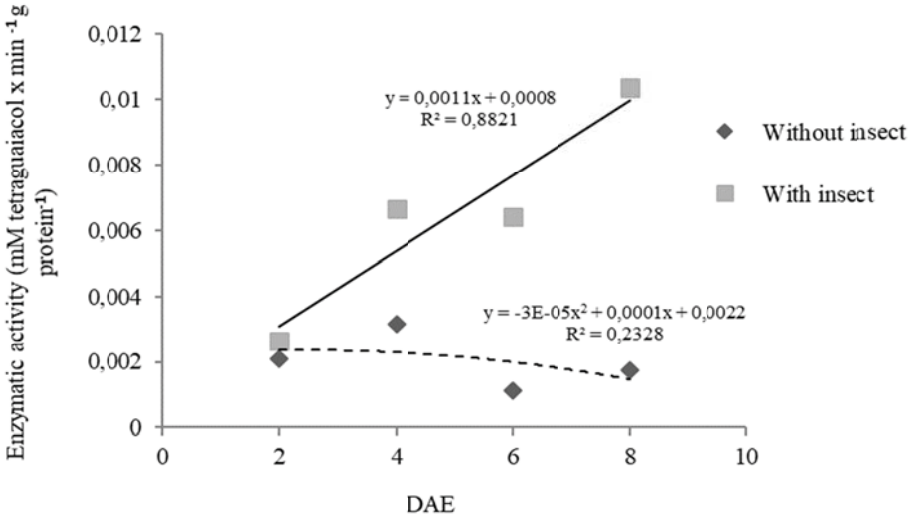

Figure 1. Peroxidase activity versus the plant seedling age separated according to the presence and absence of stink bug *Diceraeus melacanthus* on different days after emergence (DAE)

Although it has been proposed in our studies to determine the response of corn plants to the presence of *D. melacanthus* and the relationship with the activity of catalase, it was observed that this enzyme did not follow the same behavior observed did for enzyme POD, presenting extremely low levels that could not be quantified. That the stress caused during the presence of *D. melacanthus* did not activate the production of reactive oxygen forms which are commonly catalyzed by the CAT enzyme.

Table 2. Comparison of the effect of presence and absence of the stink bug *Diceraeus melacanthus* in relation on variables plant height (HT), root length (RL), dry mass of aerial part (DMAP), dry root mass (RDM) and peroxidase enzyme (POD) at 2nd, 4th, 6th and 8th days after emergence of corn plants

Variables	Days After Plant Emergence								Total Averages	
	2nd		4th		6th		8th			
	Presence	Absence	Presence	Absence	Presence	Absence	Presence	Absence	Presence	Absence
	---------- Means compared for the presence and absence of *Diceraeus melacanthus* ----------									
HT	3.52±0.08 b	4.66±0.32a	4.44±0.23b	5.88±0.46a	5.80±0.12a	6.30±0.36a	4.68±0.58b	6.40±0.26a	4.61±0.24b	5.81±0.22a
RL	8.32±0.73a	6.08±0.98a	7.54±1.15a	5.90±0.47a	10.36±0.93a	10.54±1.14a	11.0±1.72a	9.48±0.75a	9.30±0.64a	8.00±0.61a
DMAP	0.07±0.01a	0.08±0.005a	0.16±0.03a	0.14±0.02a	0.23±0.01a	0.21±0.01a	0.26±0.02a	0.29±0.02a	0.17±0.02a	0.18±0.02a
RDM	0.05±0.009a	0.05±0.003a	0.06±0.008b	0.11±0.02a	0.14±0.008b	0.21±0.01a	0.28±0.05a	0.26±0.01a	0.13±0.02a	0.16±0.02a
POD	0.003±0.0006a	0.002±0.0002a	0.007±0.002a	0.003±0.0004b	0.006±0.001a	0.001±0.0003b	0.010±0.001a	0.002±0.0004b	0.006±0.0008a	0.002±0.0002b

Note. The same letters in a line indicate no statistical differences between by Tukey's test at 5% probability.

4. Discussion

Herbivorous insects impact on plants, mainly in crops of economic interest. It is known that insect pests can negatively influence the development of cultivated plants. According to the results obtained in this research, the infestation with the stink bug *D. melacanthus* influenced the development of corn seedlings, and induced the production of defensive O_3 molecules by plants, which is demonstrated by the presence of catalyst enzymes.

The reduction in plant height can be considered as reflection of the injury caused by stink bugs. In the present study, it was observed that the green-belly stink bug caused damage to corn plants, resulting in a decrease in plant growth, in particular those plants which were kept with the stink bugs for a longer time (Table 2).

The direct damage caused by sucking insects, as observed for the green-belly stink bug in corn plants, are not notice inicitly, because they are related to internal changes in plant tissues, as compared to the damage caused by chewing insects. Even so, stink bugs can cause direct damage characterized by the suction of cellular content and release of toxic compounds during salivation, which can cause physiological changes in the plant (Lucini, 2017).

The feeding process of *D. melacanthus* was studied by Lucini and Panizzi (2016), who demonstrated that this pest had two distinct feeding characteristics, both direct feeding of xylem-carrying vessels and by inoculation of salivary enzymes and cell disruption. The saliva of these insects has toxic enzymes that lead to deformation and death of plant tissues, one of the possible causes of the reduction in plant height, as reported by Rosa-Gomes et al. (2011).

Corn plants infested by the stink bug *D. melacanthus,* between phenological stages V1 and V3 show significant damage when compared to older plants (Fernandes et al., 2020). Taking into acount our results, it appears that seedlings at 2nd DAE, phenological stage V1, which were infested by the stink bug *D. melacanthus* showed a reduction in height of approximately 23% when compared to plants that were not fed on by the bug. These results prove that the management of *D. melacanthus* is necessary right after the emergence of the plants, or even preventively, through seed treatment.

Stink bugs' presence influence on plant height was also found by Panizzi et al. (2016). According to the data of this study, the infestations of *D. furcatus* at the initial development stage of the wheat crop reduced the height of the plants. Likewise, Crosariol Netto et al. (2015) obtained results that indicated a reduction in the height of corn plants subjected to *D. melacanthus* feeding. The same authors also observed that the decrease was more striking in conventional corn hybrids, as compared to transgenic hybrids.

The presence of *D. melacanthus* did not lead to change in the mean values of the root length and DMAP of the corn plant, it was noted that the observed values did not vary from those obtained for plants kept isolated and without the injury caused by the pest (Table 2). Similar results were obtained by Roza-Gomes et al. (2011) who also found no significant difference in the DMAP for plants infested with *D. melacanthus*. The non-significant effect of the stink bug's presence on the length of the root system may be related to the initial root length of the corn seedlings. In this case the seminal roots evaluated during the experiment tend to have a slower growth immediately after emergence of the corn seedling (Magalhães et al., 2002), thus, the plants of the present experiment, which were evaluated until the eighth day after emergence, did not show any change from the the insects, in these variables.

However, the results of a slower increase of root mass obtained in this study could indicate that there was a negative effect of the photoassimilate transport from the aerial part to the root. The slower increse of root mass

could have occurred due to the imbalance between the photosynthetic production and consequent reduction in the translocation of nutrients to the root system. This process was determined by Souza and Barbosa (2015), who noticed that plants in a stressful situation tended to be less efficient photosynthetically, due to the high concentration of electrons inside the cells that combined with oxygen form the ROS.

The damage caused by the stink bug, *D. melacanthus,* manifested in the corn plants and generated oxidative stress and a concomitant increase in the concentration of ROS, verified by the action of the enzyme POD (Table 2).

The activity of the POD enzyme was altered by the presence of the bug, and from the data obtained in this study it could be stated that the activity of POD in healthy plants remained unchanged during the evaluation period (Table 2 and Figure 1).

The changes in the levels of reactive oxigens are the first responses of plants in relation to insect herbivory and beginning of plant's defense system, folowing ativation of antioxidant enzymes activity (Bi & Felton, 1995). These oxidative molecules can be harmful to plants when produced for long periods and in great intensity, as they are responsible for the peroxidation of membrane lipids and degradation of small molecules such as proteins and DNA (Huang et al., 2019). In addition, they negatively affect herbivorous insects by causing oxidative damage to the intestinal cells and reducing the absorption of the ingested nutrients (Bi & Felton, 1995).

Several studies have shown the degree of oxidative stress in plants after infestation by phytophagous insects (Ni et al., 2001; Taggar et al., 2012; Kaur et al., 2014). This was determined by the analysis of the antioxidant enzymes. Our results showed that in the longest period of coexistence of the pest and plant, 8th DAE, the plants infested with the stink bug showed that the expression of the POD enzyme was five times higher than plants without the bug. Similar results were obtained by Ni et al. (2001), these authors observed that feeding the aphid *Diuraphis noxia* (Mordw.) (Hemiptera: Aphididae) increased the activity of the POD enzyme in plants of wheat 'Halt' (*Triticum aestivum* L., Poaceae) and barley 'Morex' (*Hordeum vulgare* L., Poaceae). When compared to the control, the increase in POD activity was nine times higher in barley and three times in wheat, nine days after the initial infestation by the pest.

The quantification of antioxidant enzymes can be a way of measuring the degree of resistance of plants to insects, as genotypes that already show indiced resistance to attack by the pest may present a greater activity of antioxidant enzymes (Bi & Felton, 1995; Heng-Moss et al., 2004). Results by Kaur et al. (2014) indicated that after infestation of the *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuideae) a caterpillar, in pigeon pea genotypes (*Cajanus cajan* L. Millsp., Fabaceae) the activity of the POD enzyme was increased, showing higher values in resistant genotypes.

The increase in POD enzyme activity was also reported by Taggar et al. (2012) in black grass genotypes (*Vigna mungo* L. Hepper, Fabaceae) subjected to stress conditions by the feeding whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae). Moreover, the enzyme activity was even higher in the resistant genotypes, the high enzyme activity negatively influenced the development of the pest, and genotypes with high POD activity had fewer nymphs and whitefly adults (Taggar et al., 2012). Our results also demonstrate similar responses of corn seedlings in relation to infestation by *D. melacanthus*, increasing the activity of the enzyme POD. These findings pave the way for further studies on the negative effect of plant responses on the development of insect pests.

Through the quantification of the POD enzyme it was possible to observe that the biotic stress caused by the stink bug increased with increasing time of infestation with the stink bugs, i.e., the longer the insect fed on the plants, the greater the expression of the POD enzyme. However, this effect was not noticed in the non-infested control plants, in which the activity of the enzyme was practically constant over time, proving the effect of the stink bug in the expression of the POD enzyme. Therefore, the earlier the stink bug *D. melacanthus* is controlled in the field, the lower the possible expenses of the plant in the production of defensive compounds.

Both POD and several other antioxidant enzymes can be used to identify the stress suffered by the plant through insect injury, as demonstrated by Hanaka et al. (2018). The authors observed an increase in the expression of the enzymes superoxide dismutase, catalase, ascorbate peroxidase, and guaiacol peroxidase in corn plants, after a biotic stress caused by the infestation of the stink bug, *Trigonotylus caelestialium* (Kirkaldy) (Hemiptera: Miridae). Plants were attacked by the stink bugs had higher levels of antioxidant enzymes in their leaves and roots, when compared to plants not occupied with the stink bugs.

The activity and concentration of the CAT enzyme can vary according to the stress suffered by the plant (Vasconcelos et al., 2009). The CAT enzyme is very efficient in removing high levels of H_2O_2, but not so suitable for low concentrations of this molecule (Nicholls et al., 2000). This relationship may explain the

non-activity of this enzyme observed in the present study, probably due to the low level of production of H_2O_2 molecules, even though the plant had suffered stress; in this case, the POD enzyme was efficient in degrading the H_2O_2 produced.

The greater the activity of antioxidant enzymes, the greater the concentration of ROS in the plant, that is, the plant's stress is greater due to the attack of the pest or another factor that stimulates stress. The study of intracellular ROS concentration and the activity of POD and other antioxidant enzymes can be a tool to quantify the biotic stress of plants, due are influenced by herbivory caused by insects. The Hemiptera being the largest group of insects studied, due to the difficulty to observe and measure the damage caused by these pests (Nascimento & Barrigossi, 2014).

5. Conclusion

The injury caused by *D. melacanthus*, in addition to affecting the development of corn seedlings, as reduced plant height, they also interfered with the accumulation of RDM and caused biotic stress to plants, which could be quantified by the POD enzyme.

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Physiological Indicators in Two Lettuce Cultivars Under Saline Stress

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Abstract

Lettuce (*Lactuca sativa* L.) is considered as the main leafy vegetable in Brazil. In the last decades, there had been many changes in the predominant varietal types in the country, however, issues regarding the use of saline water inhibit the growth by the osmotic effect. The aim of this study is avaliate the effect of water salinity on physiological in lettuce cultivars. The experiment was carried out at the Alagoas Federal University, Arapiraca *Campus*, in a completely randomized design and with a 5 × 2 factorial scheme, with six replications. Five treatments of water salinity levels were analyzed (ECw: 0.14, 1.54, 2.94, 4.34, and 5.74 dS m^{-1} at 25 °C) in two types of lettuce crops (Saia Véia and Vitoria Verdinha). Stomatal conductance, net photosynthesis, transpiration rate, water use efficiency, leaf temperature, and green index were assessed at 10, 20, and 30 days after the application of the treatments. The saline stress caused by the increase in saline concentrations decreased the photosynthesis and transpiration rates, which were associated with the reduction of stomatal conductance in both cultivars. Nevertheless, Saia Véia cultivar was higher tolerance in all tested saline levels compared to Vitória Verdinha. The green index for Vitoria Verdinha was seven times higher when compared to Saia Véia from the lowest to the highest saline levels. The cultivars differ in salt sensitivity, which could be useful for producers to choose the cultivar that is most adapted to the region and breeders regarding improvement prospects for adaptation of the lettuce under saline stress. In addition to osmotic stress, which is the first to happen, there are others.

Keywords: *Lactuca sativa* L., plant ecophysiology, salinity

1. Introduction

Soil salinity and irrigation water have been arousing interest since they are regarded as worldwide issues in agricultural production. It is estimated that soils affected by salt occupy about 10% of land surface and 50% of irrigated land in the world (Ruan et al., 2010). The effects of salinisation can be observed in numerous vital ecological and non-ecological soil functions. Drivers of salinisation can be detected both in the natural and man-made environment, with climate and the foreseen climate change also playing an important role. Global annual losses in agricultural production due to salt-affected soils can be as high as US$ 12 billion (Qadir et al., 2008; Flowers et al., 2010). The salinization process is due to environmental characteristics and anthropic actions (Daliakopoulos et al., 2016). Natural characteristics include the transport of salt sediments from salinized areas to unsalted sites; actions of ascent by capillarity of the soils to surface; high rates of evapotranspiration, among other factors (Pedrotti, 2015; Walter et al., 2018).

Soil salinity, whether natural or human induced, is a major geo-hazard in arid and semi-arid landscapes. In agricultural lands, it negatively affects plant growth, crop yields, whereas in semi-arid and arid non-agricultural areas it affects urban structures due to subsidence, corrosion and ground water quality, leading to further soil erosion and land degradation (Abuelgasim & Ammad, 2019).

The Brazilian Semi-arid (BSA) region known as the Sertão is located in the Northeast part of the country and is one of the two areas most affected by climate change in Brazil (Seddon et al., 2016). The Sertão is characterized by the unique Caatinga biome - mostly consisting in deciduous forests, with uneven rainfall patterns and land distribution, climatic variation and social disparities. Several public policies have been adopted since 1877 to address the issues of the region, predominantly related to water scarcity; yet, the Sertão remains marginalized. Family farmers are the area's most vulnerable social group, in particular diffuse farmers (Machado & Revere, 2018). In these regions, agricultural activity has been a great challenge, especially for family farmers who do not have technical assistance and, plant empirically, leafy vegetables that are highly sensitive to soil salination (Qin et al., 2010; Medeiros et al., 2016).

Among the leafy vegetables sensitive to soil salinity, lettuce stands out (*Lactuca sativa* L.), consumed worldwide and is an important crop of agrosystems in semi-arid regions (Ribeiro et al., 2003). Saline environments are mainly located in coastal zones, but they can also be found inland. Natural causes for inland salinization are the presence of salt-rich rainwater or saline groundwater, high evaporation and low precipitation. In such regions, upward-directed soil water movement by capillary rise is a dominant process and rates of precipitation are too low to leach out salts. This leads to an accumulation of salts within the topsoil or at the soil surface. Typically, such conditions are found in arid regions (Colombani, Mastrocicco, & Giambastiani, 2015; Walter et al., 2018).

Accumulation of salts in the soil can alter the water uptake by plants, reducing assimilation and nutrient utilization (Parida & Das, 2005; Tavakkoli et al., 2012). The accumulation of Na^+ and/or Cl^- in chloroplasts, affects important biochemical and photochemical processes involved in photosynthesis and gas exchange (Xu & Mou, 2015; Amorim et al., 2010). Plants on the basis of adaptive evolution can be classified roughly into two major types: the halophytes (that can withstand salinity) and the glycophytes (that cannot withstand salinity and eventually die). Majority of major crop species belong to this second category. Thus salinity is one of the most brutal environmental stresses that hamper crop productivity worldwide (Flowers, 2004; Munns & Tester, 2008). In addition, the tolerance a crop has to salinity can vary between genotypes of the same species as well as the plant's development stage (Gheyi et al., 1997).

According to the researchers Gupta & Huang (2014) a comprehensive understanding on how plants respond to salinity stress at different levels and an integrated approach of combining physiological tools with and biochemical techniques are imperative for the development of salt-tolerant varieties of plants in salt-affected areas. Recent research has identified various adaptive responses to salinity stress at physiological levels, although mechanisms underlying salinity tolerance are far from being completely understood. Lettuce is considered 'moderately sensitive' to salinity and its potential yield is reached when the electrical conductivity of the saturated extract reaches the threshold value of 1.3 dS m^{-1}, with a 13% reduction in yield per unit increase of salinity above the value (Ayers & Westcot 1991). In this context, this paper aims evaluate the effect of water salinity on physiological in lettuce cultivars.

2. Methods

2.1 Environment and Meteorological Parameters

The experiment was carried out in a greenhouse with 50% shading and anti-UV treatment, located at the Alagoas Federal University (UFAL), Arapiraca *Campus* (latitude 09°41′53.6″S, longitude 36°41′26.3″W and 244 m altitude) Northeast of Brazil. The outside meteorological parameters in the greenhouse obtained from the university's automatic weather station (maximum, minimum temperature, precipitation and global radiation). The inside meteorological parameters in the greenhouse: photosynthetically active radiation obtained from spectroradiometer (Apogee, model SS-110) which measures received by pots were performed in two moments: 7:00 a.m. to 11:00 a.m. and 12:00 p.m. to 4:00 p.m. The temperature and humidity were measured with the aid of a thermo-hygrometer (model HT-208) and it was installed at the center of the protected environment, 1.0 m above from the ground level.

The climate of the region is of the AS tropical type (rainy with a dry summer), according to the classification of Köppen (1948) and Dubreuil et al. (2018). It features two well established climatic seasons: a warm and dry summer, with occasional rainfall (September to March), and wet and rainy winter (April to August). The mean annual precipitation ranges from 750 to 1000 mm (Xavier & Dornellas, 2005).

2.2 Lettuce Varieties

Sáia véia: The cycle of 35 to 40 days after transplantation. Optimum germination range: 10-27 °C. Plant with moderately wrinkled, large, and elliptical smooth leaves, with light green color. It does not form a head and has white seeds.

Vitória verdinha: The cycle of 35 to 40 days after transplantation. Optimum germination range: 10-27 °C. It has smooth leaves, slightly wrinkled and soft texture, dark green in color. It does not form a head and has white seeds.

These two lettuce cultivars used in this experiment are the most planted in the APL region (Aranjo Produtivo Local) in the municipality of Arapiraca in Northeast of the Brazil. The APL is reference in the production of fruits and vegetables and it distributes to consumer markets in Brazil.

2.3 Soil Analyses

A soil sample of 1.0 kg was collected at the UFAL area in a 0-0.20 m layer that represents the effective depth of the lettuce root system. Samples were sent for chemical and physical analyses, respectively, to the Soil Fertility Laboratory and Soil Physics Laboratory (Department of Environmental Resources, School of Agronomic Sciences, UNESP, Botucatu, São Paulo).

Soil analysis classified it as a sandy loam soil and showed the following features: pH in water of 6.3; 18 mg/dm^3 of P; 3.6 mmol$_c$/dm^3 of K; 44 mmol$_c$/dm^3 of Ca; 16 mmol$_c$/dm^3 of Mg; 0 mmol$_c$/dm^3 of Al; 11 mmol$_c$/dm^3 of H$^+$ Al; 17 mg/dm^3 of Fe; 4.7 mg/dm^3 of Mn; 0.5 mg/dm^3 of Cu; 1.4 mg/dm^3 of Zn; 25 mmol$_c$/dm^3 of S; 63 mmol$_c$/dm^3 of base sum (BS); 85% base saturation (V), 74 mmol$_c$/dm^3 cation exchange capacity (CEC) and 18 g/dm^3 organic matter (OM). The physical analyzes quantified the levels of sand (484 g/kg), silt (62 g/kg), and clay (162 g/kg), soil with a medium texture.

2.4 Water Analyses

The water (control) used in the experiment had the following elements: 3 mg/L of N; 0.3 mg/L of P; 12 mg/L of K; 5 mg/L of Ca; 3 mg/L of Mg; 4 mg/L of S; 4.6 mg/L of Na; 0.12 mg/L of B; 0 mg/L of Cu; 0 mg/L of Fe; 0 mg/L Mn and 0 mg/L of Zn.

The electrical conductivity curves were obtained as a function of sodium chloride (NaCl) concentration, in which the desired electrical conductivity (dS m^{-1}) was multiplied by 640 mg L^{-1}, according to Richards (1954). The different saline levels were obtained by dissolving sodium chloride (NaCl) in water, that was obtained from the supply system of UFAL, in buckets of 14 L. Saline water that was used for treatments irrigation was verified before each irrigation with the aid of a Portable Digital Conductivity Meter (Mod. Cd-4301).

2.5 Experimental Design

The experiment was assessed in three periods at interval of 10 days. Saline treatments were applied three days after the seedlings were established, thus starting the first evaluation period. The experimental design was completely randomized, arranged in a 5 × 2 factorial scheme (five salt levels and two lettuce cultivars), with six replicates. The experimental unit was represented by a pot with a plant. The treatments resulted from the combination of five irrigation water salinity levels, as follows: 0.14 (control); 1.54; 2.94; 4.34; and 5.74 dS m^{-1}. The effect of irrigation water salinity levels on the analyzed variables was measured through the analysis of variance, whose results of the treatments were studied through polynomial regression analysis. The effect of the cultivars was compared by the Tukey's test at 0.05 probability. Statistical tests were performed using the SISVAR software, version 5.3Build 77 (Ferreira, 2014).

2.6 Cultivations Conditions

Initially, seedlings of lettuce (*Lactuca sativa* L.) of the Saia Véia and Vitória Verdinha varieties were produced in Styrofoam trays with 200 cells, filled with the Bioplant substrate. Seedlings were irrigated daily with water (electrical of conductivity of 0.14 dS m^{-1}) and every 7 days were fertigated (0.5% of urea in the trays). The transplanting was performed manually 30 days after sowing (DAS), where seedlings that showed more vigor were selected and transplanted into plastic containers and one plant was conditioned per pot, with seedlings with four to six leaves. As a form of irrigation control, plastic vats with a capacity of five liters were drilled and connected to collecting hoses to conduct the fraction of drained water. Each pot had in the bottom filled with 500 g of gravel (3 cm high) and the remaining volume was completed with soil, totaling 5.5 kg. Pots were placed on a bench at 1.0 m of distance from the ground level.

The beginning of the experiment was characterized by raising the soil to the field capacity; for this, recipients were saturated with water and wrapped individually in plastic, to force the loss of water only by drainage. When the drainage ceased, the plastic covers were removed and the recipients were weighed on a digital scale (0.1 g precision), thus obtaining the control weight, corresponding to the field capacity, which was approximately 6.8 kg for each recipient; finally, seedlings were transplanted.

After the beginning of treatments, irrigation was performed twice a day (at 8:00 am and 4:00 p.m.), based on the water consumption of the plants of the previous day, keeping the soil moisture close to the field capacity.

The estimated water volume for daily irrigation was divided by the factor 0.9, to restore soil moisture to field capacity, also, to obtain a leaching fraction (FL) of 10%, using the following formula: (VI-VD)/(1-FL), where VI: is the volume of water to be applied on the irrigation; VA: is the volume of water applied in the previous irrigation, and VD: is the volume of drained water in the previous irrigation (VI, VA, and VD were described in mL).

2.7 Physiological Analysis

The physiological analysis was performed with an infrared gas analyzer IRGA (LI 6400, LICOR, Lincoln, USA), with a photosynthetic photon flux density (PPFD) according to the environment of 800 μmol photons m^{-2} s^{-1}. Analyses were performed between 8:00 a.m. and 10:00 a.m., at 10, 20, and 30 days after the application of treatments (DAAT). In fully expanded and non-senescent leaves, the following measures were performed: net photosynthesis (*A*-μmol CO_2 m^{-2} s^{-1}) and transpiration (*E*-mmol H_2O m^{-2} s^{-1}) rates, stomatal conductance (*gs*-H_2O m^{-2} s^{-1}), leaf temperature (T °C), and water use efficiency (WUE-μmol CO_2 $mmol^{-1}$ H_2O) through the *A*/*E* ratio. The green index (GI) was measured in 10 fully expanded and non-senescent leaves. For each leaf, 10 readings were collected, and a mean of the green index was obtained. The readings were obtained with a Portable Chlorophyll Meter (Model SPAD-502®, Soil Plant Analyzer Development, Minolta, Japan).

3. Results and Discussion

3.1 Data From the Automatic Meteorological Station

The recorded, for the greenhouse proximal area, where the pots were allocated with the seeds, from germination to the end of the plants' biocycle, temperatures that ranged from 24 to 36 °C (Figure 1A) and global radiations that ranged from 5 to 28 MJ m^{-2} day^{-1} (Figure 1B). Inside the greenhouse, the photosynthetically active radiation was measured, ranging from 558 μmol m^{-2} s^{-1} from 7:00 a.m. to 11:00 a.m. and 678 μmol m^{-2} s^{-1} from 12:00 p.m. to 4:00 p.m. The temperature inside the greenhouse ranged from 40 °C (May) to 28 (June) and relative humidity ranged from 30 to 80%.

The precipitation average for the year of this experiment is in line with the averages recorded for the region, as can be seen in the author's paper Xavier and Dornella (2005), in which municipality of Arapiraca has a rainfall regime concentrated in the autumn-winter period, confirming the regional dynamics. Most of your rainfall precipitates in just 3 months (usually, May, June and July).

In the month in which the experiment started (May), the highest temperature of the year was recorded, a fact that repeated itself at the end of November (Figure 1A), which increased the internal temperature of the greenhouse, as well as the relative humidity. Temperature and relative humidity influence the metabolic functions, transpiration, and mechanisms of thermal control of plants (Teruel, 2010).

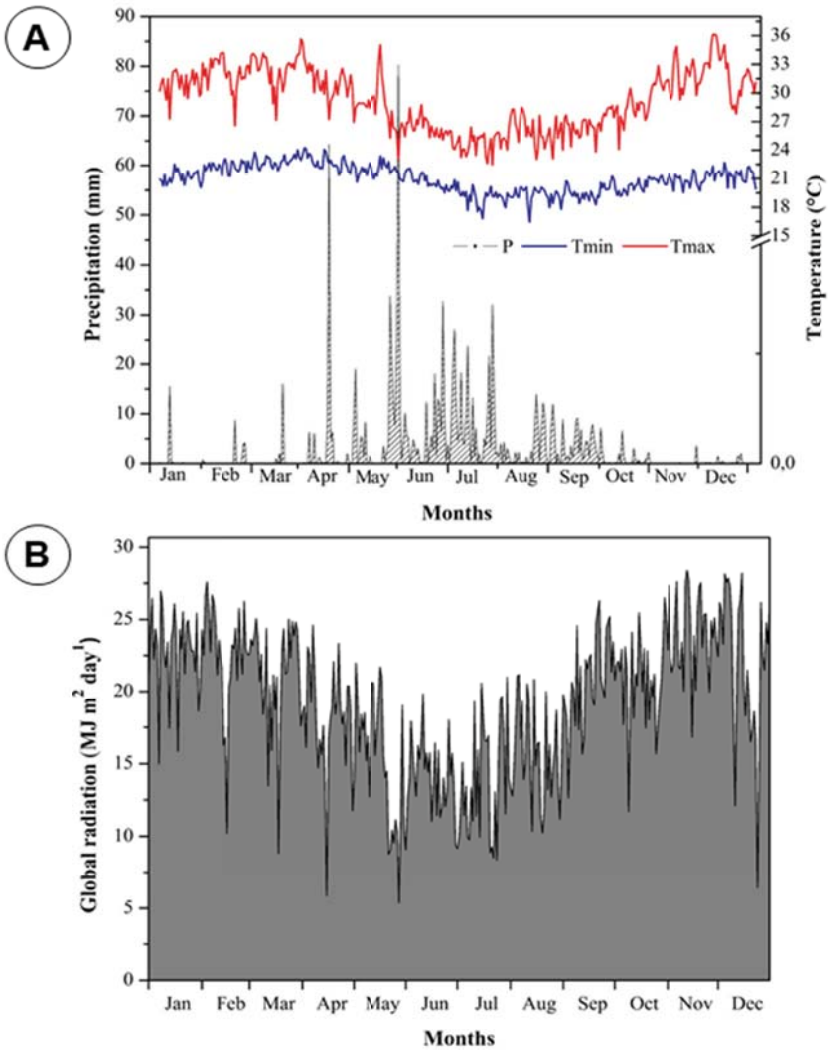

Figure 1. Climatic data obtained from the automatic weather station. A- Maximum and minimum temperatures and precipitation levels recorded in the city of Arapiraca. B- Global radiation registered in the outside area of the greenhouse. Year 2016

3.2 Physiological Parameters

There was a significant effect of irrigation water salinity during the gas exchanges in the two lettuce cultivars. However, there was no interaction between salinity and cultivars. Regarding transpiration rates (*E*) in both cultivars, the interaction effect was observed only at 20 DAAT (Table 1).

Table 1. Summary of variance analysis for photosynthesis (*A*), transpiration (*E*) and stomatal conductance (*gs*) for Saia Véia and Vitória verdinha cultivars, as a function of the electrical of conductivity water) of irrigation, at 10, 20 and 30 days after application of treatments (DAAT)

Sources of Variation	GL	Mean Squares (DAAT)								
		A			*E*			*Gs*		
		10	20	30	10	20	30	10	20	30
Salinity (S)	4	1.25 **	2.30 **	3.62 **	1.38 **	2.49 **	3.42 **	0.0001 **	0.0070 **	0.0005 **
Linear Regression	1	4.90 **	9.16 **	14.20 **	5.48 **	3.85 **	13.46 **	0.0009 **	0.0249 **	0.0020 **
Quadratic Regression	1	0.01 NS	0.01 NS	0.22 **	0.02 NS	0.06 **	0.18 **	0.0000 NS	0.0020 NS	0.0000 NS
Deviation regression	2	0.04 NS	0.02 NS	0.03 NS	0.01 NS	0.04 *	0.03 NS	0.0000 NS	0.0005 NS	0.0000 NS
Cultivar (C)	1	0.01 NS	1.31 **	1.11 NS	0.15 NS	1.15 **	0.12 **	0.0015 **	0.0366 **	0.0000 NS
S × C	4	0.01 NS	0.05 NS	0.01 NS	0.34 NS	0.02 *	0.01 NS	0.0000 NS	0.0034 NS	0.0000 NS
Residual	10	0.02	0.03	0.02	0.01	0.01	0.01	0.0000	0.0009	0.0000
CV (%)		0.81	0.89	0.71	1.94	1.06	1.16	0.70	8.39	1.46
Salinity (S)		------- µmol CO_2 m^{-2} s^{-1} -------			------ mmol H_2O m^{-2} s^{-1} ------			---------- mol H_2O m^{-2} s^{-1} ----------		
S1 (0.14 dS m^{-1})		16.96	19.02	20.76	6.60	7.74	8.56	0.3074	0.4201	0.5217
S2 (1.54 dS m^{-1})		16.66	18.45	20.45	6.29	7.00	8.07	0.3029	0.3601	0.5144
S3 (2.94 dS m^{-1})		16.41	18.10	19.86	5.98	6.64	7.58	0.2957	0.3511	0.5120
S4 (4.34 dS m^{-1})		15.85	17.61	19.10	5.49	6.14	7.04	0.2922	0.3230	0.5000
S5 (5.74 dS m^{-1})		15.61	17.05	18.45	5.15	5.69	6.18	0.2894	0.3141	0.4937
Cultivar (C)										
C1 (Saia Véia)		16.32a	18.30 a	19.72 a	5.93 a	6.88 a	7.56 a	0.3061 a	0.3965 a	0.5094 a
C2 (Vitória Verdinha)		16.27 a	17.79 b	19.72 a	5.87 a	6.40 b	7.41 b	0.2889 b	0.3109 b	0.5073 a
MSD		0.13	0.16	0.14	0.11	0.07	0.09	0.0021	0.0295	0.0074

Note. (*) Significant at 0.05 and (**) at 0.01 probability; (NS) Not Significant; (MSD) Minimum Significant Difference. Means followed by the same letter do not differ from each other, in columns, by the Tukey test at $p < 0.05$ level.

On the other hand, when the isolated effect of salinity levels was analyzed, there was an effect ($p < 0.01$) for the three variables (*A*, *E*, and *gs*) in the different periods assessed, which highlights the direct influence of saline water on growth and development of the two lettuce cultivars (Table 1).

The presence of NaCl in the irrigation water influenced the photosynthesis of the assessed cultivars only at 20 DAAT, where the cultivar C1 showed an increase of approximately 3% in this variable compared to C2. Compared to C2, cultivar C1 showed higher values for *E* at 20 (7%) and 30 (2%) DAAT and *gs* at 10 (5.6%) and 20 (21.6%) DAAT (Table 1).

Saline stress caused linear reductions in *gs* in all assessed periods; however, at 20 DAAT, the decrease was more expressive with approximately 5% per unit increase of ECw. The decrease of the lowest (0.14 dS m^{-1}) to the highest saline level (5.74 dS m^{-1}) was 24.7% (Figure 2A).

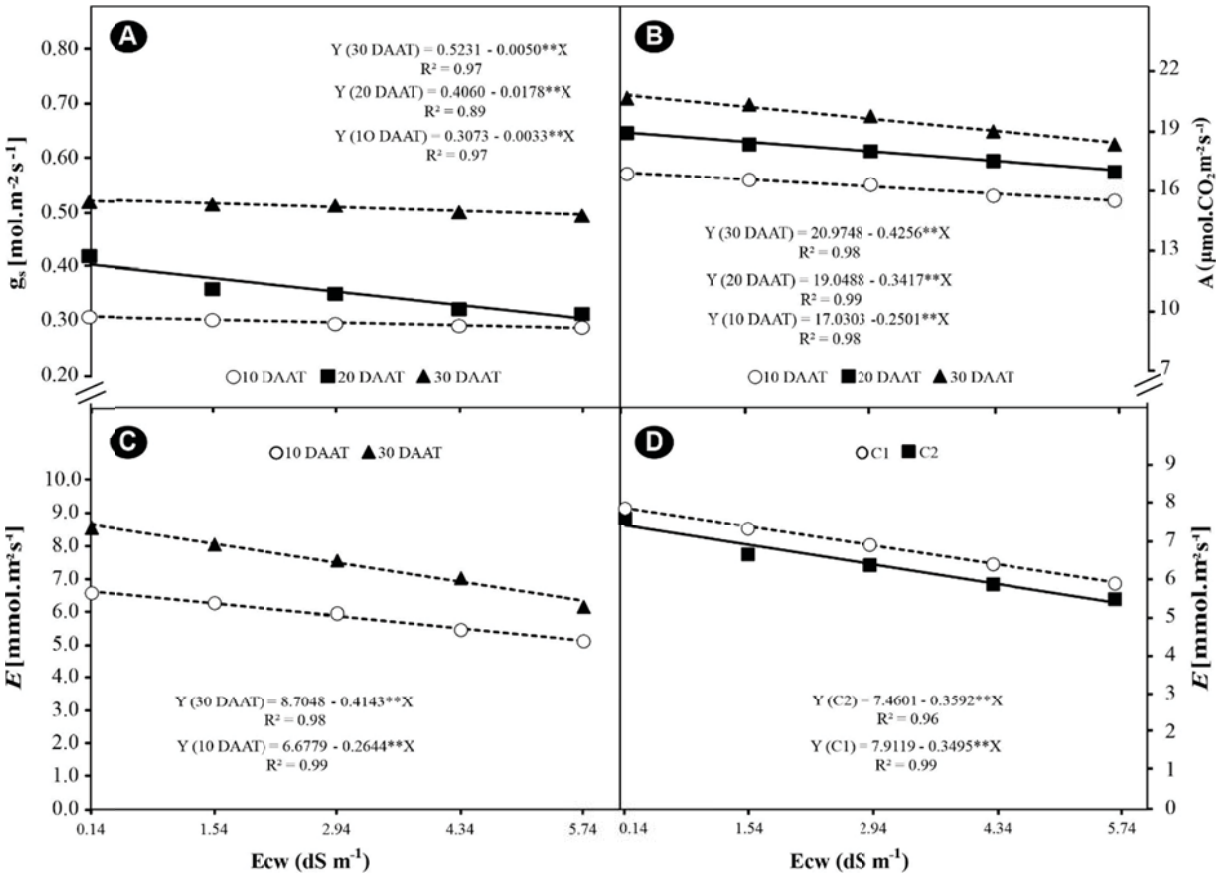

Figure 2. Physiological parameters of two lettuce cultivars submitted to increasing salinity levels. (A) Stomatal conductance, (B) photosynthesis, (C) Transpiration only for salinity levels and (D) Transpiration only cultivars effect

In the photosynthesis analysis, there were linear reductions of 1.5, 1.8, and 4.8%, at 10, 20, and 30 DAAT, respectively (Figure 2B), for each increased unit of ECw. The decrease in the lowest level concerning higher saline levels was 8.2, 10, and 26.8% at 10, 20, and 30 DAAT, respectively.

The transpiration rate at 10 and 30 DAAT was only effective for the salinity levels, with linear reductions of 4.1 and 4.8%, respectively, for each increased unit of ECw (Figure 2C). The decrease in the lowest level concerning the highest saline level was 22.3 and 26.8%, respectively, at 10 and 30 DAAT.

At 20 DAAT, cultivars presented linear adjustments, with reductions of 4.5 and 4.9% with a unit increment of salinity, respectively. The decrease between the lowest and highest saline levels was 24.9 and 27.2% for C1 and C2, respectively (Figure 2D).

In the two cultivars evaluated, the rates of *A* and *E* were directly influenced by *gs*. Under conditions of stress, especially water and salt, the stomatal closure can be seen as a positive response of the plant to the maintenance of water balance (Yousifet al., 2010). It is well known that salt stress reduces root hydraulic conductivity resulting in decreased water flow from roots to shoot, even in osmotically adjusted plants (O'Leary, 1969; Prisco & Gomes-Filho, 2010). This decrease in water flow due to salt stress may cause a lowering in leaf water content, that would result in stomatal closure in order to maintain their water status (Azevedo-Neto et al., 2004). Thus, *gs* may have been reduced due to lower water availability of the roots (Tatagiba et al., 2014).

Similar results were observed by Han and Lee (2005) in which lettuce plants submitted to salt stress showed severe reductions in *A*, *E*, and *gs*. Similar results were found by Moles et al. (2016), where severe restrictions on *A* and *E* rates were observed in two tomato cultivars submitted to different saline levels.

Water use efficiency (WUE) and leaf temperature (T) were not influenced by the interaction of salinity levels and cultivars (Table 2); however, Green Index (GI) was significant only at 30 DAAT.

Table 2. Summary of variance analysis for Green Index (GI), Water Use Efficiency (WUE), and Leaf Temperature (LT) of cultivars Saia Véia and Vitória verdinha, as a function of the electrical conductivity of water of irrigation at 10, 20, and 30 days after application of the treatments (DAAT)

Sources of Variation	GL	Mean Squares (DAAT)								
		GI			WUE			LT		
		10	20	30	10	20	30	10	20	30
Salinity (S)	4	1.67 **	11.23 **	11.64 **	0.14 **	0.17 **	0.18 **	1.84 **	2.27 **	4.78 **
Linear Regression	1	6.64 **	42.03 **	42.03 **	0.54 **	0.69 **	0.68 **	6.78 **	8.61 **	18.53 **
Quadratic Regression	1	0.02 NS	0.35 NS	3.81 **	0.01 NS	0.00 NS	0.03 **	0.00 NS	0.37 *	0.17 NS
Deviation regression	2	0.00 NS	1.28 **	0.36 *	0.00 NS	0.00 NS	0.01 *	0.28 NS	0.04 NS	0.20 *
Cultivars (C)	1	0.04 NS	73.35 **	46.82 **	0.00 NS	0.08 **	0.02 **	1.88 **	0.02 NS	0.03 NS
S × C	4	0.04 NS	0.20 NS	1.80 **	0.01 NS	0.00 NS	0.00 NS	0.14 NS	0.02 NS	0.02 NS
Residual	10	0.05	0.09	0.05	0.00	0.00	0.00	0.17	0.05	0.04
CV (%)		0.75	1.01	0.74	2.32	1.25	1,53	1.25	0.67	0.61
Salinity (S)		--- Dimensionless greatness ---			------ CO_2 mmol^{-1} H_2O ------			---------- °C ----------		
S1 (0.14 dS m^{-1})		29.95	27.58	29.03	2.57	2.46	2.42	32.39	32.53	32.71
S2 (1.54 dS m^{-1})		29.58	28.00	29.40	2.65	2.64	2.54	33.08	32.79	33.43
S3 (2.94 dS m^{-1})		29.23	30.18	30.30	2.74	2.73	2.62	33.38	33.04	33.67
S4 (4.34 dS m^{-1})		28.75	30.65	31.05	2.89	2.87	2.71	33.45	33.77	34.77
S5 (5.74 dS m^{-1})		28.33	31.38	33.33	3.03	3.00	2.99	34.26	34.36	35.45
Cultivars (C)										
C1 (Saia Véia)		29.21 a	27.64 b	29.09 b	2.77 a	2.68 b	2.63 b	33.00 b	33.33 a	33.97 a
C2 (Vitória Verdinha)		29.12 a	31.47 a	32.15 a	2.78 a	2.80 a	2.69 a	33.62 a	33.27 a	34.04 a
MSD		0.22	0.30	0.23	0.06	0.01	0.04	0.42	0.22	0.21

Note. (*) Significant at 0.05 and (**) at 0.01 probability; (NS) Not Significant; (MSD) Minimum Significant Difference. Means followed by the same letter do not differ from each other, in columns, by the Tukey test at $p < 0.05$ level.

When analyzing the isolated effect of salinity levels ($p < 0.01$) for the three variables (GI, WUE, and T), the different evaluation periods were observed, and interaction was observed between salt levels and cultivars exclusively for GI at 30 DAAT. The cultivar factor was significant ($p < 0.01$) at 20 and 30 DAAT. Regarding cultivars C1 and C2, higher values were observed for GI 13.9 and 10.5% at 20 and 30 DAAT, respectively (Table 2).

The variable WUE was influenced by the presence of NaCl at 20 and 30 DAAT, where cultivar C2 increased by 4.5 and 2.3%, respectively, when compared to C1 (Table 2). At 10 DAAT, LT was higher for C2, which presented an increase of 1.9% about C1 (Table 2).

At 10 DAAT, each increased unit of ECw showed a linear decrease of 1% for the outcome GI; this decrease reached 5.3% when the lowest and highest saline levels were compared (Figure 3A). At 20 DAAT, plants presented a linear increase of 1.5% at each unit increase of ECw; this increase reached 14.7% when the lowest and highest saline levels were compared (Figure 3A).

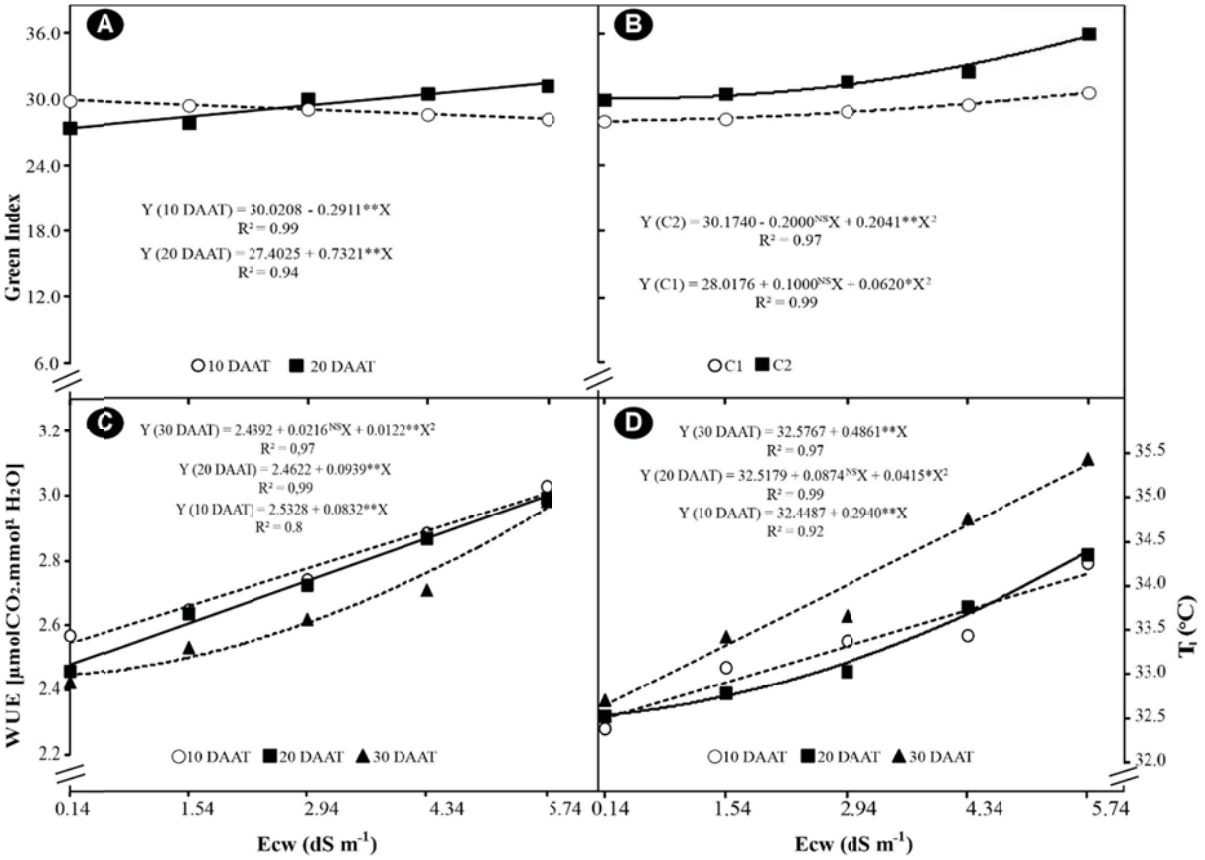

Figure 3. A and B: Green Index (GI), C: Water Use Efficiency (WUE), D: Temperature (T) as a function of the electrical conductivity of the irrigation water, at 10, 20, and 30 days after application of the treatments (DAAT)

At 30 DAAT, the outcome GI was effective ($p < 0.01$) for the interaction between the salinity levels and the cultivar, in which a linear increase occurred for each increased unit of ECw in C1 and C2 of 2.2 and 3.4%, respectively; also, an increase in GI in C1 and C2 of 9.4 and 19.1% occurred, respectively, from lowest to highest saline levels (Figure 3B).

The intensity and duration of stress progression influence the plant's responses to water scarcity and salinity since these factors influence the processes associated with acclimatization (Chaves et al., 2009). The increase in GI of the plants at 20 DAAT compared to 10 DAAT, suggests acclimatization of the plants to the different levels of NaCl.

The increase in GI shows that the plant can use mechanisms such as a greater thickness of the mesophyll and an increase in the number of thylakoids and chloroplasts, which indicate activation in the process of protection to the photosynthetic apparatus (Lacerda et al., 2006).

Some studies showed that increases in GI were observed in response to salt stress in *Vigna unguiculta* L. (Lacerda et al., 2006) and *Arachis hypogaea* L. (Graciano et al., 2011); on the other hand, *Vigna unguiculta* L (Praxedes et al., 2009) showed a reduction in GI, which might be attributed to a weakening of the pigment-protein complex in most sensitive cultivars due to exposure to salinity (Taffouo et al., 2009).

WUE rates were inversely proportional to A and E at all assessments, confirming the effects of saline stress with linear increases at all periods (Figures 2B, C, D, and 3C). There was a unit increase of ECw of 3.3, 3.8, and 3.9% for 10, 20, and 30 DAAT, respectively. Regarding the lowest and highest saline levels, values of 18.3, 21.3, and 21.8% were obtained at 10, 20, and 30 DAAT, respectively (Figure 2C).

The increase in the influence of salt levels to WUE was reported for two tomato cultivars (Moles et al., 2016) and Citrus plants (Syvertsen et al., 2010) under different saline levels. This evidence sustains the hypothesis that an increase in WUE, under salt stress, can be interpreted as a mechanism of tolerance to NaCl in lettuce plants.

The increase in the electrical conductivity of water promoted linear increases in leaf temperature in all evaluated periods (Table 2). However, at 30 DAAT, there was an increase for each unit increase of ECw of 1.5%, with a rise in temperature from the lowest to the highest salt levels of 8.3%, equivalent to 2.7 °C (Figure 2D).

In Figure 4, it is possible to observe at 30 days after the application of saline levels the aspect of each cultivar and it is noticeable that the cv 'Vitória Vedinha', has superior green index (visual aspect), however, the cv 'Saia Véia' has with physiological performance higher.

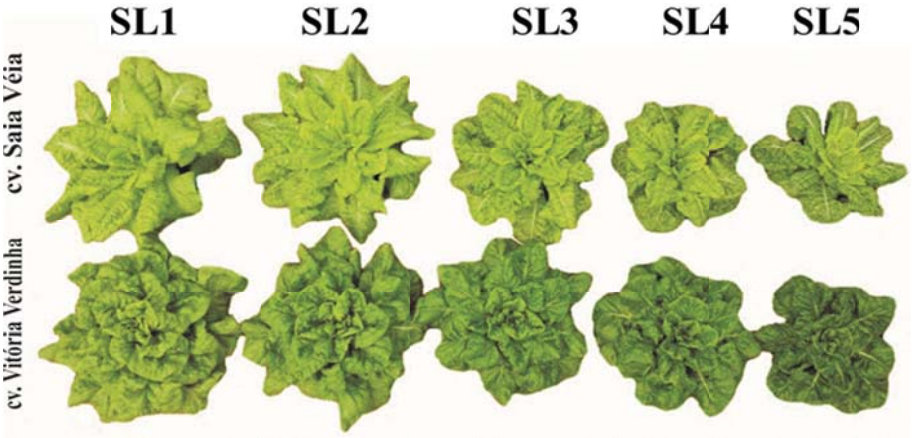

Figure 4. Morphological aspect of the two varieties of lettuce submitted to different saline levels 30 days after treatments. SL: Saline Level. 1 = 0.14; 2 = 1.54; 3 = 2.94; 4 = 4.34 and 5 = 5.74 dS m^{-1}

The results of the present study were similar to those described by Viana et al. (2004), who observed that an increase in salinity may promote leaf temperature elevation, since by osmotic effect, the restriction of the water flow towards the soil - plant - atmosphere and, consequently, transpiration, resulting in elevation of LT.

4. Conclusion

The cv. Saia Véia was higher values of gs, *A*, and *E*, whereas cv. Vitoria Verdinha showed higher tolerance to salt stress, indicated by the higher values of WUE and GI in different levels of NaCl. Results from the present study highlight the need for new studies regarding the genetic improvement of the species so breeders can find genes that can be cloned and later incorporated into new extremely productive varieties, that are not yet sensitive or not tolerant to different levels of saline soil or irrigation water.

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Reaction of Sweet Cassava Genotypes to *Xanthomonas phaseoli* pv. *manihotis* From Three Regions of Brazil

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Abstract

Xanthomonas phaseoli pv. *manihotis* (*Xpm*) is the causal agent of Cassava Bacterial Blight (CBB), one of the most important cassava diseases. The aim of this study was to evaluate the susceptibility of sweet cassava genotypes to strains of *Xpm* from three different geographic regions of Brazil in greenhouse conditions. The inoculation of 14 genotypes of cassava was made by cutting leaflets using scissors previously soaked in a bacterial suspension with 1×10^8 UFC ml^{-1} and also by inserting a soaked toothpick into the bud of the oldest leaf. The results showed significant differences when the cassava genotypes were individually evaluated in relation to the *Xpm* strains used; however, the relationship between cassava genotypes and *Xpm* strains was significant for wilt symptom. The UnB 1111 strain was more aggressive than the UnB 1386 strain based on the average value of the reaction grade, showing the variation that exists between the bacterial isolates from different regions. Considering the reaction of cassava germplasm's resistance to the three strains used in the study, the BGMC 434 genotype was the only one classified as resistant based on the average reaction grade. The genotypes BGMC 753, BGMC 1289, BGMC 982 and the elite clones BRS 396, BRS 397, BRS 398, 259/08 and BRS 399 were classified as moderately resistant, which indicates the possibility of recommending them for disease favorable regions.

Keywords: bacteriosis, genetic resistance, *Manihot esculenta* Crantz, plant breeding, variability

1. Introduction

Cassava, *Manihot esculenta* Crantz (Euphorbiaceae), is a perennial shrub grown mainly in tropical countries. It is important in the food security of these populations due to its robustness, which is reflected in the capacity to store high amounts of starch in its roots under conditions in which other species would not even survive (Filgueiras & Homma, 2016). Brazil is the 5th largest world producer of cassava (FAOSTAT, 2019), with an area of 1,362,185 hectares and a production of 18,994,242 tons (IBGE, 2020). However, the productive potential of the crop can be limited by the attack of pests and diseases, so protection against these is a crucial element in the production of cassava (Fukuda et al., 2002; Ceballos et al., 2004).

One of the main diseases that affect the crop in cultivation areas in the Center South region of Brazil is the bacteriosis known as Cassava Bacterial Blight (CBB) (Fukuda et al., 2002) caused by *Xanthomonas phaseoli* pv. *manihotis* (*Xpm*) (Constantin et al., 2016). In conditions favorable to the development of the disease, with sudden temperature fluctuations between day and night above 10 °C and annual precipitation above 1200 mm, root yield losses can vary from 30 to 100% (Anjos et al., 2013).

The first symptoms of bacteriosis in the Cerrado region of Central Brazil usually appear at the beginning of the rainy season, after the dry season, and show their maximum at the peak of the rainy season. The infected plants

initially show wilting on the young leaves, irregular watery spots on the leaflets, symptoms of a systemic nature, such as canker and exudation of gum on the stems and descending death of the leaves, culminating in the death of the plant. The bacterium invades the plant systemically through the xylem vessels, which are responsible for transporting water and mineral salts (Hillocks & Wydra, 2002; Anjos et al., 2013).

The spread of the pathogen in the same planting area occurs through rain, which disperses bacterial cells present in the exudation of infected plants to healthy plants. The penetration of bacteria in healthy plants occurs through natural openings and wounds present in the leaves. The spread of the bacterium over long distances occurs through the planting of contaminated seeds, especially in new areas where the disease is not yet present. When the propagation material is contaminated by the bacterium *Xpm*, sprout losses of more than 25% can occur (López, 2002; Anjos et al., 2013).

Differences in virulence were found between *Xpm* isolates in Africa and Latin America, which showed different speeds in the appearance of bacteriosis symptoms, due to the variation in aggressiveness between the isolates (Restrepo et al., 2004; Portz et al., 2006; Nery-Silva et al., 2007; Banito et al., 2010; Ogunjobi et al., 2010, Martin et al., 2017). Recent transcriptome studies indicate that the various responses of accessions to *Xpm* may result from the action of TALEs—Transcription Activator Like Effectors (Ramirez et al., 2020).

Since the *Xpm* bacterium has a systemic habit, curative control methods are not viable. The use of resistant varieties is the most efficient control method, even in climatic conditions favorable to the development of the epidemic, considering that they suffer fewer losses from the disease (Fukuda et al., 2002; Ceballos et al., 2004; Nery-Silva et al., 2007, Martin et al., 2017).

The objective of the study was to evaluate the reaction of genotypes of sweet cassava to isolates of *Xanthomonas phaseoli* pv. *manihotis* from three geographic regions of Brazil under greenhouse conditions.

2. Method

2.1 Cassava Accessions Used

To carry out the study, six accessions of table cassava (Table 1) conserved in the Regional Collection of Cassava of the Cerrado (BGMC) were selected, and these were: (i) three varieties recommended for cultivation in the Federal District and Surrounding Region, BGMC 982 (IAPAR 19), BGMC 753 (IAC 576-70) and BGMC 1289 (BRS Moura) (Fialho et al., 2009; Vieira et al., 2009, 2011b, 2018); (ii) the variety recommended for cultivation in the region of the Recôncavo Baiano and the Coastal Tablelands, BGMC 1398 (BRS Dourada); (iii) the 'Taquari' variety (BGMC 434) used as a standard for bacteriosis resistance; (iv) the 'Vassourinha' variety (BGMC 962) used as a standard for susceptibility to bacteriosis. In addition to these six accessions, eight elite sweet cassava clones from the Embrapa Cerrados genetic improvement program were also used in the study (BRS 399, BRS 396, BRS 397, 215/08, 259/08, 83/08, BRS 398 and 26/08).

Table 1. Genotypes and genealogy of cassava accessions evaluated in this study

Genotypes	Genealogy
26/08	BGMC 751 × BGMC 753
83/08	BGMC 751 × BGMC 753
215/08	BGMC 751 × BGMC 753
246/08	BGMC 751 × BGMC 753
259/08	BGMC 1289 × BGMC 753
BRS 396	BGMC 1289 × BGMC 753
BRS 397	BGMC 1289 × BGMC 753
BRS 398	BGMC 1218 open pollination
450/08	BGMC 1218 open pollination
BRS 399	BGMC 1218 open pollination
BGMC 753 (IAC 576-70)	IAC Ouro do Vale open pollination
BGMC 982 (IAPAR 19)	Landrace (local variety)
BGMC 1289 (BRS Moura)	Landrace (local variety)
BGMC 1398 (BRS Dourada)	Landrace (local variety)
'Taquari' (BGMC 434)	Landrace (local variety)
'Vassourinha' (BGMC 962)	Landrace (local variety)

The plants were obtained by the method of rapid propagation (Rodrigues et al., 2008) with some adaptations, where approximately 10 cm seedlings were planted in plastic vases of 2-liter capacity, filled with substrate that served as a propagation chamber, which aims to increase the temperature and relative humidity inside the chamber, stimulating the production of shoots. After the shoots reached approximately 10 cm in height, they were cut and placed for rooting in sterile water. The rooted shoots were transplanted individually into plastic bags suitable for seedlings, which were 10 cm wide, 20 cm high and 0.07 cm thick; these were filled with an autoclaved mixture composed of red latosol, sand, tanned bovine manure and vermiculite, in the following proportions 2:1:1:1, in addition to which 40 liters of the mixture and 100 grams of the NPK fertilizer formulation (4-14-8) were added. The transplanted seedlings were placed on concrete benches inside the greenhouse for acclimatization.

The vigorous seedlings of the accessions produced by the method of rapid propagation were selected uniformly, with height between 25 and 30 cm. These were transplanted into plastic vases of 2 L capacity, using the autoclaved substrate in the proportions previously described. After transplantation, the seedlings were placed on concrete benches inside the greenhouse. The greenhouse was covered with a shade screen with 30% shade, in order to reduce the direct incidence of sunlight on the plants and the temperature inside the greenhouse. One week after transplantation, plants were inoculated with the three strains of *Xpm* used in the study, plus the control with distilled water.

2.2 Plant Pathogenic Bacteria Tested

Three strains of *Xpm* from three regions of Brazil were used: UnB 1386 (Planaltina-DF), UnB 1152 (Manaus-AM) and UnB 1111 (Paranavaí-PR), preserved in the collection of the Bacteriology Laboratory of the Department of Phytopathology of the Institute of Biological Sciences of the University of Brasília.

2.3 Bacterial Inoculation

The bacterial suspension was prepared using new *Xpm* cultures, grown in 523 medium (Kado & Heskett, 1970) for 48 h. The strains of the bacterium were diluted in sterile distilled water and the suspensions measured in a spectrophotometer with a wavelength of 550 nm until absorbance 0.350 was obtained, which corresponds to the concentration of 1×10^8 CFU ml^{-1}.

The inoculation of the leaves was performed by prior immersion of small, sterile scissors in the bacterial suspension and subsequent cutting of three leaflets in different leaves. Sterile toothpicks were used to inoculate the stem, immersed in the bacterial suspension for 10 minutes. One toothpick per plant was inserted into the axial side of the oldest leaf.

The monitoring of the temperature and relative humidity of the air inside the greenhouse was performed by a datalogger (ITLOG-75), programmed to record the hourly parameters.

2.4 Evaluation of Cassava Accessions to Cassava Bacterial Blight

The assessment was made based on the scale of Ramos and Takatsu (1987), with modifications where the symptoms of the aerial part were measured through the quantification of leaf spots and wilting, the systemic infection was measured through the quantification of regrowth with or without descending death, appearance of bacterial pus on the stems and descending death (Table 2). The symptoms of the aerial part were evaluated individually according to the variation in the speed of appearance over the weeks (Table 2). For the intervals of variations in the onset of symptoms, scores were assigned, which ranged from 1 to 5 (Table 2).

The relationship between the symptoms was determined by the average of the notes of the symptoms of spot (S), wilt (W) and complex of symptoms of systemic nature (CSSN), which is called degree of reaction (DR). After obtaining the averages of the grades represented by the DR, the classification of the reaction of the cassava accessions to *Xpm* was performed using the variations of the DR (Table 2).

Table 2. Scales of grades attributed to the different intervals of appearance of leaf spots, wilt and symptoms of systemic nature, in plants inoculated with *Xanthomonas phaseoli* pv. *manihotis* (adapted from Ramos & Takatsu, 1987) and resistance and susceptibility ranges, based on the degree of reaction (DR) related to the symptoms of bacteriosis

Grades	**Strain symptoms**
1	No symptoms of the disease
2	Appearance of leaf spots typical of the disease from 4 weeks after inoculation
3	Appearance of leaf spots typical of the disease in the 3rd week after inoculation
4	Appearance of leaf spots typical of the disease in the 2nd week after inoculation
5	Appearance of leaf spots typical of the disease in the 1st week after inoculation
Grades	**Wilt symptoms**
1	No symptoms of the disease
2	Appearance of wilted leaves typical of the disease from the 5th week after inoculation
3	Appearance of wilted leaves typical of the disease in the 4th week after inoculation
4	Appearance of wilted leaves typical of the disease in the 3rd week after inoculation
5	Appearance of wilted leaves typical of the disease in the 2nd week after inoculation
Grades	**Symptoms of a systemic nature**
1	No symptoms of the disease
2	Partial recovery without descending death from the apex of the plant
3	Partial recovery with descending death from the apex of the plant
4	Presence of bacterial exudation along the stem without or with partial recovery
5	Descending death of the plant, with the presence of exudate and without partial recovery
DR	**Classification regarding resistance and susceptibility, based on the degree of reaction (DR)**
1.0-2.0	Resistant (R)
2.1-3.0	Moderately resistant (MR)
3.1-4.0	Moderately susceptible (MS)
> 4.1	Susceptible (S)

2.5 Statistical Analysis

The experimental design used was completely randomized (CDR), in a factorial scheme composed of 14 genotypes of cassava and three strains of *Xpm*, with five replicates for each treatment. The data were first transformed into the square root of X and later submitted to joint variance analysis. The means were compared using the Tukey test at 5% probability of error. All statistical analyses were performed with the aid of the SAS statistical program (Statistical Analysis System-version 9.1.3) (SAS, 2006).

3. Results

The results of the joint analysis of variance revealed the existence of significant differences at 5% probability of error, in relation to the average scores of the individual symptoms of S, W, CSSN and DR, between the genotypes evaluated for the three strains of *Xpm* used in the study (Table 3). This revealed the existence of genetic variability regarding the resistance of genotypes to *Xpm*. As for strains, only significant differences were detected at 5% probability of error between strains for W and DR, showing that the strains evaluated show different degrees of aggressiveness when these characters are considered (Table 3). However, the presence of significant interaction between the genotype and strain effects was detected only for W. This indicates that the order of classification of the genotypes regarding the symptoms of W was influenced by the strain effect, as the accessions presented different responses to the strains. This can be explained by genetic variations in both genotypes and strains.

Table 3. Summary of the analysis of joint variance and coefficient of variation (CV%) of the symptoms of spots (S), wilt (W), complex of symptoms of a systemic nature (CSSN) and the degree of reaction (DR) of 14 genotypes of table cassava inoculated with three isolates of *Xanthomonas phaseoli* pv. *manihotis*

SV*	DF**	MS***			
		S	W	CSSN	DR
Genotypes (G)	13	0.0269*	0.0770*	1.5108*	0.4599*
Isolates (I)	2	0.0008	0.5066*	0.1203	0.0821*
Interaction G x I	26	0.00032	0.0586*	0.0386	0.0105
Residue (R)	168	0.0022	0.0147	0.0876	0.0129
Total	209				
CV (%) ****		2.32	9.32	24.37	6.58

Note. * SV = Sources of Variation; ** DF = Degrees of Freedom; *** MS = Mean Square; **** CV = Coefficient of Variation.

The average temperature in the evaluation period was 23.9 °C, with a maximum of 30.2 °C and a minimum of 16.4 °C. The average relative air humidity was 86.8% in the period of the research, with a maximum of 99.9% and a minimum of 49.9%.

Through the average of the degree of reaction (DR), it was possible to separate the cassava genotypes into four groups of resistance to *Xpm*. Considering the three strains of *Xpm*, a difference was observed regarding the reaction of the cassava genotypes, which allowed them to be grouped into resistance classes (Table 4).

Table 4. Comparison of averages of 14 genotypes of table cassava inoculated with three isolates of *Xanthomonas phaseoli* pv. *manihotis*, by means of individual symptoms of wilt (W), spot (S), systemic symptom complex (CSSN) and the degree of reaction (DR) and the classification as to the degree of resistance in resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S), through individual symptoms

Genotype	W			UnB 1386, UnB 1111 and UnB 1152			General Reaction
	UnB 1386	UnB 1111	UnB 1152	S	CSSN	DR	
BGMC 962	4.58 aA*	5.00 aA	5.00 aA	4.33 ab**	4.20 a	4.45 a	S
83/08	4.80 aA	4.41 aA	4.00 abcA	4.00 c	2.25 b	3.46 b	MS
BGMC 1398	4.58 aA	5.00 aA	5.00 aA	4.58 a	2.62 b	3.96 ab	MS
26/08	4.16 abA	4.80 aA	4.58 abA	4.00 c	2.04 b	3.42 b	MS
BRS 397	3.96 abA	3.96 aA	3.17 bcA	4.08 bc	1.25 c	2.86 c	MR
BRS 396	3.96 abA	4.41 aA	3.53 abcA	4.00 c	1.14 c	2.86 c	MR
BGMC 1289	3.96 abA	3.96 aA	3.17 bcA	4.00 c	1.00 c	2.69 c	MR
BGMC 753	3.76 abcA	4.58 aA	3.57 abcA	4.00 c	1.17 c	2.86 c	MR
215/08	3.50 abcB	4.58 aA	2.96 cB	4.00 c	1.00 c	2.69 c	MR
BRS 398	3.39 abcA	4.20 aA	3.17 bcA	4.00 c	1.00 c	2.66 c	MR
BRS 399	2.96 bcA	3.57 aA	3.57 abcA	4.00 c	1.00 c	2.59 c	MR
BGMC 982	2.79 bcB	4.16 aA	2.96 cB	4.00 c	1.06 c	2.59 c	MR
259/08	2.53 cB	3.57 aA	4.00 abcA	4.00 c	1.25 c	2.72 c	MR
BGMC 434	1.35 dA	1.93 bA	1.77 dA	4.00 c	1.00 c	2.00 d	R
Overall average	3.59	4.16	3.61	4.07	1.57	2.99	

Note. * = Means followed by uppercase letters in the horizontal, and lowercase letters in the vertical, differ from each other at 5% probability of error by the Tukey means comparison test; ** = Average values for the three isolates used in this experiment.

Considering the DR average in relation to the three strains used in the study, the BGMC 962 genotype was classified as susceptible to the disease, in line with its previous classification by Vieira et al. (2011a). In turn, the BGMC 1398, 26/08 and 83/08 genotypes were classified as moderately susceptible by means of the mean DR value (Table 4).

The analysis of the averages of the degree of reaction (DR) for the three *Xpm* isolates, considering the 14 cassava

genotypes, allowed us to establish the difference in aggressiveness between the strains used in the study (Table 5). From the comparison of the averages of the degree of reaction, strain UnB 1111 was more aggressive than strain UnB 1386. Strain UnB 1152 showed intermediate aggressiveness in relation to strains UnB 1111 and UnB 1386.

Table 5. Comparison of means of three strains of *Xanthomonas phaseoli* pv. *manihotis* by means of wilt (W) and degree of reaction (DR), evaluated in 14 genotypes of table cassava

Strains	W	DR
UnB 1111	4.12* a**	3.10 a
UnB 1152	3.57 b	2.96 ab
UnB 1386	3.53 b	2.86 b

Note. * = Statistical analysis performed with transformed means for square root of X and untransformed data presented in the table; ** = Means followed by distinct letters in the column differ from each other, at 5% probability of error by the Tukey means comparison test.

4. Discussion

The genotypes BGMC 753, BRS 396, BRS 397, 215/08, BGMC 1289, BRS 398, 259/08, BGMC 982 and BRS 399 were classified as moderately resistant, based on the mean DR value. The accessions BGMC 982 and BGMC 753 had already shown resistance to bacteriosis in an experiment conducted in Planaltina-DF (Vieira et al., 2011a). The accession BGMC 434 was classified as resistant, according to the average values of DR considering the three strains (Table 4).

The differences in the genetic constitution of the cassava genotypes are responsible for the classification variation after inoculation with *Xpm* strains from different regions. It is especially important to carry out studies in regions where cassava cultivars will be produced, when the objective is to select and recommend materials resistant to *Xpm*. The existence of differences in the classification of cassava genetic constitutions, using *Xpm* strains under greenhouse conditions, has already been reported (Wydra et al., 2004; Restrepo et al., 2004; Nery-Silva et al., 2007; Banito et al., 2010). In a similar study, the occurrence of a significant interaction between 26 isolates of *Xpm* and 17 genotypes of cassava was detected after the inoculation of the plant stems measured under the area of the disease progress curve (Restrepo et al., 2004). However, the interaction of isolates x cassava genotypes after leaf inoculation was not significant. Banito et al. (2010) used four highly virulent *Xpm* strains from different African regions and 24 cassava genotypes among local and improved varieties. After the inoculation of the plant stems, they found that the interaction of isolates x cassava genotypes was significant, with six groups of different genotypes being useful for identifying the pathotype, differing from the result obtained in this study for the DR.

Nery-Silva et al. (2007) used the same scale of visual symptom scores of aerial parts used in this study and evaluated ten cultivars of sweet cassava and eight clones of bitter cassava, using two strains of *Xpm*. They observed that five varieties of sweet cassava demonstrated differences regarding resistance classification, and no variety of manioc showed variation, being all classified as resistant to disease. In other studies, there were also differences in the classification of cassava genotypes regarding the resistance group after inoculation of *Xpm* isolates. After inoculation of the stem of 111 improved cassava genotypes, with suspensions 10^7 UFC mL^{-1} of four stems of *Xpm* in greenhouse conditions, Wydra et al. (2004) revealed that based on the average of the area below the disease progress curve, 16 genotypes were resistant, 26 showed moderate resistance and 69 susceptibility. Banito et al. (2010) reported that 11 cassava genotypes were classified as resistant, four as moderately resistant and nine as susceptible after the inoculation of the stems of 24 local cassava varieties with four virulent strains of *Xpm*.

When evaluating 1090 cassava accessions, composed of traditional varieties and improved in terms of the reaction of cassava germplasm to three strains of *Xpm* under greenhouse conditions, Ogunjobi et al. (2010) reported that of the 490 local varieties used in the study, 30.1% were highly susceptible, 12.3% susceptible, 24.3% tolerant and 14.3% resistant. Among the 600 improved cassava accessions, 4.3% were highly susceptible, 36.6% tolerant, 30.1% resistant and 11.1% showed high resistance. The differences in the classification of cassava accessions from the average reaction grade (DR), with the use of *Xpm* strains in the inoculation of leaves and stems, can be explained by the genetic variability between cassava accessions and *Xpm* isolates from different locations.

The cassava genotypes 215/08, BGMC 982 and 259/08 showed significant interaction with the *Xpm* strains used in the study for the symptom of W. When inoculated with strain UnB 1111, the plants of genotypes 215/08 and BGMC 982 showed quicker appearance of wilted leaves than when inoculated with strains UnB 1386 and UnB 1152. When inoculated with strains UnB 1111 and UnB 1152, plants of genotype 259/08 showed a greater speed in the appearance of wilted leaves than when inoculated with the strain UnB 1386. The variation in the speed of appearance of withered leaves of a given cassava genotype with the inoculation of different strains of *Xpm* can be explained by the genetic variability between the cassava genotypes and the difference in aggressiveness between the *Xpm* strains. Analyzing the capacity of colonization of vascular tissue after inoculation with strains of *Xpm* in genotypes of sweet and bitter cassava, Nery-Silva et al. (2007) found differences in the percentage of systemic infection with the variation of the *Xpm* strain.

Considering the 14 cassava genotypes, the analysis of the mean of the symptom of W for the three isolates of *Xpm* allowed us to establish differences in the speed of symptom manifestation throughout the evaluation period. From the comparison of averages for W, the cassava genotypes inoculated with strain UnB 1111 showed a higher rate of onset of wilted leaves than when inoculated with strains UnB 1152 and UnB 1386. A factor that may have influenced the greater speed of manifestation of the symptom of W in cassava seedlings inoculated with strain UnB 1111 is a high capacity for colonization of vascular tissue, which obstructs the passage of water and nutrients, due to the genetic characteristic of the strain. In a similar study, assessing the percentage of systemic stem infection after the inoculation of eight bitter cassava genotypes with two strains of *Xpm*, Nery-Silva et al. (2007) found that the Uberlândia strain was more efficient in colonizing vascular tissues than the Lavras strain.

In similar studies, it was possible to verify the difference in aggressiveness between the strains of *Xpm* from different regions. Through the evaluation of 21 isolates of *Xpm* in relation to aggressiveness, using the 'Verdinha" variety that is highly susceptible to the pathogen, it was possible to determine six isolates of *Xpm* as more aggressive than the others (Portz et al., 2006). Nery-Silva et al. (2007) evaluated 10 cultivars of sweet cassava in relation to the reaction to *Xpm* from the region of Lavras-MG and Uberlândia-MG. Based on the average values of the visual symptoms of the aerial part, percentage of systemic infection and percentage of defoliation, they reported the occurrence of differences in aggressiveness between the isolates employed in the study, the Lavras-MG isolate being more aggressive than that of Uberlândia-MG for the three characteristics evaluated.

Comparable results were described by Banito et al. (2010), when evaluating the aggressiveness of four *Xpm* isolates from different African regions after inoculation of the plant stems of 24 local and improved varieties of cassava. Those authors found that the Uganda 12 and GSPB 2507 strains were more aggressive than the GSPB 2511 and GSPB 2506 strains. Ogunjobi et al. (2007) evaluated the aggressiveness of 72 *Xpm* isolates in six cassava accessions. Of all the *Xpm* isolates, 10% were not aggressive for the 'Isu' variety; however, 58.5% were highly aggressive for the same variety. As for clones 30572 and 94/0430, 34% and 32.7% of the *Xpm* isolates were moderately aggressive, respectively. Both clones were resistant to 12.9% of the tested isolates. Only 44.2% of the *Xpm* isolates were highly aggressive to clone 4 (2) 1425, but it was moderately resistant to 45% of the *Xpm* strains. Clones 96/0037 and 60142 were resistant only to 8.2 and 8.8% of the *Xpm* strains, being susceptible to 58.5 and 61.9% of the strains, respectively.

In the Cote d'Ivoire, in the field, Martin et al. (2017) also observed variation in the aggressiveness of *Xpm*, suggesting that it is associated with different bacterial strains and different varieties of cassava used in the region, which present different levels of sensitivity to the pathogen. This field experiment also took place under favorable climatic conditions for the pathogen, such as alternate rainy and dry seasons, high humidity and significant temperature oscillations between day and night.

In the present study, the only genotype classified as resistant in terms of the average degree of reaction for the three strains evaluated was accession BGMC 434. The genotypes recommended for cultivation in the Federal District and Surrounding Region, BGMC 753 (IAC 576-070), BGMC 1289 (BRS Moura), BGMC 982 (IAPAR 19) and the elite clones BRS 396, BRS 397, 446/08, 259/08, 215/08 and BRS 399 were classified as moderately resistant to bacteriosis.

5. Conclusion

There were significant differences between cassava genotypes and *Xpm* strains. The UnB 1111 strain was more aggressive than the UnB 1386 strain. The BGMC 434 genotype was the only one classified as resistant in terms of the average degree of reaction for the three strains evaluated, proving its resistance pattern. The cultivars BGMC 753, BGMC 1289, BGMC 982 are already widely used by farmers in the Federal District and Surrounding Region, and they presented as moderately resistant to *Xpm* for the three strains, as did the elite clones BRS 396, BRS 397, 446/08, 259/08, 215/08 and BRS 399, presenting the potential for cultivation in

regions where climatic conditions are favorable for the development of the disease.

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Performance of Urea-Based Fertilizers Associated With Elemental Sulfur or Polymers on Ammonia Volatilization

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Abstract

High N-NH_3 losses are expected when conventional urea is applied to the soil surface. In order to reduce it, urea granules could be coated with different materials to decrease fertilizer dissolution rate or to stabilize N-NH_4^+ by acidification. In this study, we investigated the effect of a polymer-coated urea and powdered S0 added to urea, in the presence or absence of a S-oxidizing bacterium (*Acidithiobacillus thiooxidans*), on soil pH, SO_4^{2-} availability, NH_4^+, and NH_3 volatilization. Applying S^0 before urea and the inoculation with bacteria have promoted the highest S^0 oxidation rates. The greater decrease in soil pH occurred when S^0 was applied before urea at a higher dose, which also decreased NH_3 volatilization by 83% up to 4 days after urea application. However, the decrease in soil pH did not increase the concentration of NH_4^+, nor did it decrease the accumulated amount of volatilized NH_3 over time. The inoculation of *A. thiooxidans* accelerates S^0 oxidation process, but it was insufficient to counteract the H^+ consumption by urea hydrolysis. Therefore, the S^0 application with urea did not offer chemical protection against NH_3 loss, but a physical barrier in the controlled-release urea had less dissolved urea in soil and reduced NH_3 losses.

Keywords: N-fertilizer, slow-release, urease, S^0, NH_3 volatilization

1. Introduction

Urea is a solid N-fertilizer with the highest concentration of N (46%) and the lowest cost per unit of nutrient. Nevertheless, N losses by ammonia (NH_3) volatilization decrease its agronomic efficiency. In soil, urea is hydrolyzed to NH_3 and CO_2 (Sigurdarson et al., 2018), and NH_3 can be lost to the atmosphere as a gas. The acidity around the granule application region is a key driver of a lower NH_3 volatilization (Longo & José De Melo, 2005; Viero et al., 2014) because if there is sufficient H^+ in the medium, the NH_3 is converted to NH_4^+ (da Costa et al., 2019), which is a more stable N-specie in soil. Hence, the application of acidifying substances together with urea might lower the emission of NH_3 and temporarily keep a higher NH_4^+ concentration in soil (Trenkel, 2010).

Elemental sulfur (S^0) is a high-purity S-source (> 98%), and due to this, a small mass of product would be required to satisfy the ideal H^+ demand for N hydrolysis in urea granules. However, the form of sulfur absorbable by plants is sulfate (SO_4^{2-}). Thus, oxidation of S^0 is mediated by soil microbes, such as bacteria of the genus Acidithiobacillus, which produces H_2SO_4 that is readily dissociated in soil solution as SO_4^{2-} and $2H^+$ (Li et al., 2005; Kupka et al., 2009). If we consider the hypothetical reaction $CO(NH_2)_2 + S^0 + 3/2O_2 \rightarrow 2NH_4^+ + H_2CO_3 + SO_4^{2-}$, the oxidation of one mole of S should neutralize the alkalinity produced by one mole of urea; hence, the ideal mass ratio of S/N is 1.145. In controlled-release urea fertilizers, S^0 is used together with polymers as a coating on urea granules to retard the granule dissolution due to hydrophobic nature of those substances (Wang et al., 2019). However, although the polymer layer improves the granule coating quality, it limits the action of microorganisms in the S^0 oxidation (Zhao et al., 1996).

Fine particles of S^0 have faster oxidation in soil because of their high specific surface (Chapman, 1989; Friesen, 1996). However, the application of powdered S^0 results in losses by wind and poor distribution, and it might irritate the human airway (Boswell & Friesen, 1993). Alternatively, adherent substances are used to protect S^0 on

urea granules, decreasing the segregation of the mixture and maintaining the large surface of S^0 particles, which is a condition more favorable to S^0 oxidation. Moreover, applying S^0 together with urea, rather than separately, reduces the costs of fertilizer's application. However, there is little information about the effect of this association in the acidity and stability of NH_4^+ in soil. Therefore, the objective of this study was to investigate the performance of urea-based fertilizers associated with S^0 or polymer application in the presence or absence of bacteria Acidithiobacillus on the volatilization of NH_3 and stabilization of NH_4^+ in soil.

2. Method

The experiment was conducted using a forced airflow system adapted to capture volatilized NH_3. Powder elemental sulfur (S^0) was passed through a 320-mesh sieve. We tested the following: urea (45% N) with and without application of powdered elemental sulfur, an early application of S^0 and *Acidithiobacillus thiooxidans*, and commercially controlled-release urea coated with S^0, polyolefins and ethylene-vinyl acetate copolymers-EVAC (accounted for 3% of coated fertilizers weight and 37% N and 16% S).

The soil used was a Ustox Oxisol, sieved through a 1 mm sieve, containing 190 g kg^{-1} of clay, 40 g kg^{-1} of silt, 770 g kg^{-1} of sand, 160 g kg^{-1} of maximum water retention, 12 g kg^{-1} of organic matter, 12.7 mg dm^{-3} of S and 4.8 cmolc dm^{-3} of cation exchange capacity and pH 5.6.

The 12 treatments are described in Table 1. Eight treatments were obtained from the combination of two S^0 doses (0.86 and 2.29 g dm^{-3}) in powder form (< 0.053 mm), early S^0 application (12 d), or S^0-urea joint application and the presence or absence of *A. thiooxidans*. Two treatments corresponded to a commercial controlled-release urea in the presence or absence of *A. thiooxidans*. In addition, two other treatments corresponding to the application of only urea and one control, without the application of S^0. The N dose was 2 g kg^{-1}, corresponding to an S/N ratio equal to 0.43 and 1.15 for the S^0 doses 0.86 and 2.29 g kg^{-1}, respectively. Elemental sulfur and urea were applied at 0.5 cm soil depth as well as 140 µL of a suspension containing 109 mL^{-1} cells of *A. thiooxidans*. Soil samples were collected immediately before N-urea application and 4, 9, 15, and 19 d after that. A completely randomized experimental design was used. Sixty experimental units were obtained from the combination of the 12 treatments (Table 1) with the five sampling times, and we had three replications per experimental unit.

Table 1. Description of treatments

Treatments	S^0 (g dm^{-3})	TI ‡
Control	0	Control
Urea	0	U
Urea + early S^0 application †	0.86	U + S^0e
Urea + early S^0 + *A. thiooxidans* †	0.86	U + S^0ei
Urea + S^0	0.86	U + S^0
Urea + S^0 + *A. thiooxidans*	0.86	U + S^0i
Controlled release urea	0.86	CRU
Controlled release urea + *A. thiooxidans*	0.86	CRU*i*
Urea + early S^0 application †	2.29	U + S^0e
Urea + early S^0 † + *A. thiooxidans*	2.29	U + S^0ei
Urea + S^0	2.29	U + S^0
Urea + S^0 + *A. thiooxidans*	2.29	U + S^0i

The experimental units consisted of Falcon tubes (50 mL) containing 45 cm^3 of soil. Five tubes of the same treatment were grouped and put into the volatilization chambers. Soil moisture was maintained between 85 and 100% of the water retention capacity of the soil, by monitoring the weight of experimental units; room temperature was 25±2 °C.

The volatilization chambers were closed glass pots with approximately 1.5 L of internal volume. They were connected to an air inlet tube (6.25 cm^3 min^{-1}) and an air outlet pipe connected to Erlenmeyer flasks (125 mL) containing 25 mL of boric acid (20 g L^{-1}) and methyl red and bromocresol green as a color indicator for collecting NH^3 (g). To avoid potential contamination with NH_3 from the atmosphere, the airflow inlet system was filtered through a phosphoric acid solution (pH < 3.6).

Ammonia collected in the boric acid solution was titrated with HCl 0.005 mol L^{-1}. Volatilization chambers were quickly opened to collect one tube at each time of incubation for soil analyses. After the experiment, soil samples

were air-dried for pH and electrical conductivity determination in a soil suspension:water (ratio 1:2.5), NH_4^+-N (Kempers & Zweers, 1986), NO_3-N (Cataldo et al., 1975), and SO_4^{2-}-S (Hoeft et al., 1973).

The results were submitted to analysis of variance and the treatments were compared within each time by the Tukey test at 5% of probability. We calculated the Pearson linear correlation coefficients for the variables NH_4^+, pH, SO_4^{2-}, accumulated NH_3, and rate of NH_3 volatilization using the software R version 3.2.0. We adjusted equations through linear and nonlinear models for accumulated NH_3 using the *Stats* package of the software R.

3. Results

3.1 NH_3-Volatilization

There were contrasting differences between treatments in terms of NH_3 volatilization (Table 2, Figure 1). In fact, the accumulated of N-NH_3 volatilization for up to 19 d corresponded to 65% of the total N applied as urea, 56% for urea combined with the application of powdered S^0, regardless of the application time or dose of S^0, and 3% for the controlled-release urea. On average, NH_3 volatilization was 95% lower for the controlled-release urea than conventional urea.

Table 2. Adjustment of sigmoidal and linear equations for the percentage of accumulated NH_3-N (g) as a function of incubation time (t).

Treatment	Dose of S^0 (g dm^{-3})	Equation	Estimated parameters			R^2
			n	*b*	$t_{50\%}$	
U	0	$\hat{y} = \frac{n}{1+\left(\frac{1}{10}\right)^{\frac{1}{b}(t-t_{50\%})}}$	62.78	2.513	6.5	0.98
U + S^0*ei*	0.86		57.63	2.072	6.8	0.99
U + S^0*e*	0.86		49.38	2.007	6.0	0.99
U + S^0*i*	0.86		54.62	2.022	5.2	0.98
U + S^0	0.86		57.09	2.297	6.3	0.99
CRU*i*	0.86	$\hat{y} = bt$	$0.04235^{(0.10)}$			0.72
CRU	0.86		0.10758^{**}			0.69
U + S^0*ei*	2.29	$\hat{y} = \frac{n}{1+\left(\frac{1}{10}\right)^{\frac{1}{b}(t-t_{50\%})}}$	55.76	1.365	7.8	0.99
U + S^0*e*	2.29		53.99	1.947	6.6	0.99
U + S^0*i*	2.29		52.38	2.149	6.0	0.96
U + v	2.29		54.87	1.971	6.0	0.98

Note. For sigmoidal equations: NH_3-N maximum (n); maximum rate of NH_3 volatilization (1/b), dag kg^{-1} d^{-1}; days for 50% of NH_3-N maximum ($t_{50\%}$). For linear equations: (**) and (0.10) indicate significance at 1 or 10% by t-test. U = Urea; U + S^0*ei* = Urea + early S^0 + *A. thiooxidans*; U + S^0*e* = Urea + early application; U + S^0*i* = Urea + S^0 + *A. thiooxidans*; U + S^0 = Urea + S^0; CRU*i* = Controlled release urea + *A. thiooxidans*; CRU = Controlled-release urea.

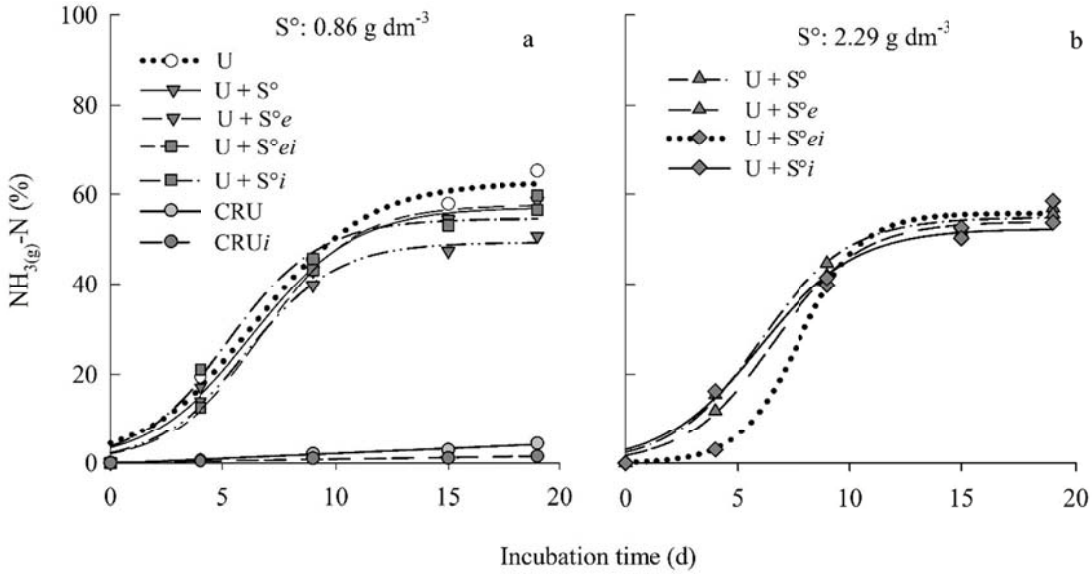

Figure 1. Accumulated NH_3-N, as a percentage of urea-N applied, estimated by sigmoidal and linear equations. Treatments: urea (U); urea + application of elemental sulfur (U + S^0); urea + early application of elemental sulfur at 12 d (U + S^0*e*); urea + early application of elemental sulfur and *A. thiooxidans* at 12 d (U + S^0*ei*); urea + application of elemental sulfur and *A. thiooxidans* (U + S^0*i*); controlled-release urea (CRU) or controlled-release urea + application of *A. thiooxidans* (CRU*i*)

The maximum percentage of NH_3 loss estimated by sigmoidal and linear models ranged from 63 (U) to 49% (U + S^0*e*; 0.86 g kg^{-1} S^0) of N applied (Table 2, Figure 1). The highest rates of NH_3 volatilization were 7.3 g kg^{-1} d^{-1} for the U + S^0*ei* treatment and the lowest was 4.4 g kg^{-1} d^{-1} for the U + S^0 treatment (Table 2).

The application of S^0 (0.86 or 2.29 g kg^{-1}) and urea at the same time, regardless of inoculation, had no significant effect on NH_3 volatilization. Comparisons between treatments, not including controlled-release urea, highlighted U + S^0*ei* (at 2.29 g kg^{-1} of S^0) by promoting a dramatic reduction in NH_3 volatilization for up to 9 d after incubation (Figure 1).

3.2 NH_4^+-N in Soil

The controlled-release urea (CRU and CRU*i*) had a more gradual release and hydrolysis; consequently, the concentration of NH_4^+ in soil was lower than (50%) other treatments, up to day 9 after N application (Figure 2). However, the concentration of NH_4^+ in the soil gradually increased until day 19, when there were no differences in NH_4^+ concentrations in the soil.

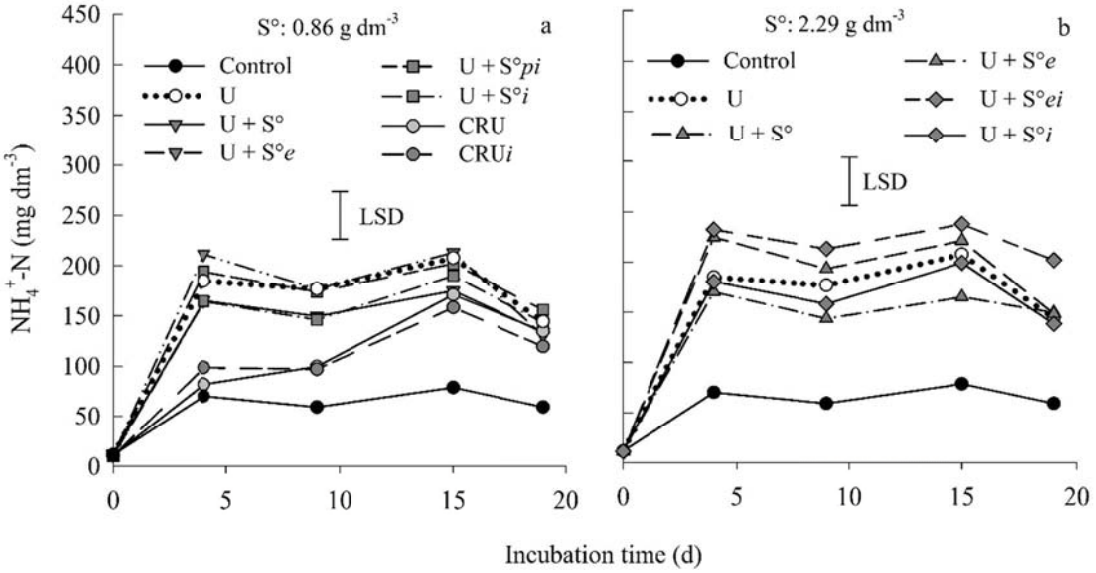

Figure 2. Concentration of NH_4^+-N in soil. Treatments: control without fertilizer application; urea (U); urea + application of elemental sulfur (U + S^0); urea + early application of elemental sulfur at 12 d (U + S^0*e*); urea + early application of elemental sulfur and *A. thiooxidans* at 12 d (U + S^0*ei*); urea + early application of elemental sulfur and *A. thiooxidans* (U + S^0*i*); urea protected with elemental sulfur and polymer coating (CRU) or urea protected with elemental sulfur and polymer coating + application of *A. thiooxidans* (CRU*i*). Vertical bars indicate the least significant difference (LSD = 47.54) between treatments (Tukey test, p = 0.05)

3.3 SO_4^{2-}-S in Soil

There were significant effects of treatments on the SO_4^{2-}S concentration in soil. When S^0 was applied earlier, in the presence of *A. thiooxidans* (U + S^0*ei*), SO_4^{2-}-S concentrations reached higher values (Figure 3), also demonstrating on contrasting S^0 doses. In fact, for U + S^0*ei* treatment, the concentrations of SO_4^{2-}-S in soil were 51 and 167 mg dm^{-3} for 0.86 and 2.29 g dm^{-3} S^0, respectively, which corresponded to the recovery of 6 and 7% of the total S^0 applied, for low and high S^0 doses, respectively. For other treatments containing S^0, there were no significant increases in the concentrations of SO_4^{2-}-S in soil, even under the inoculation with *A. thiooxidans* (Figure 3).

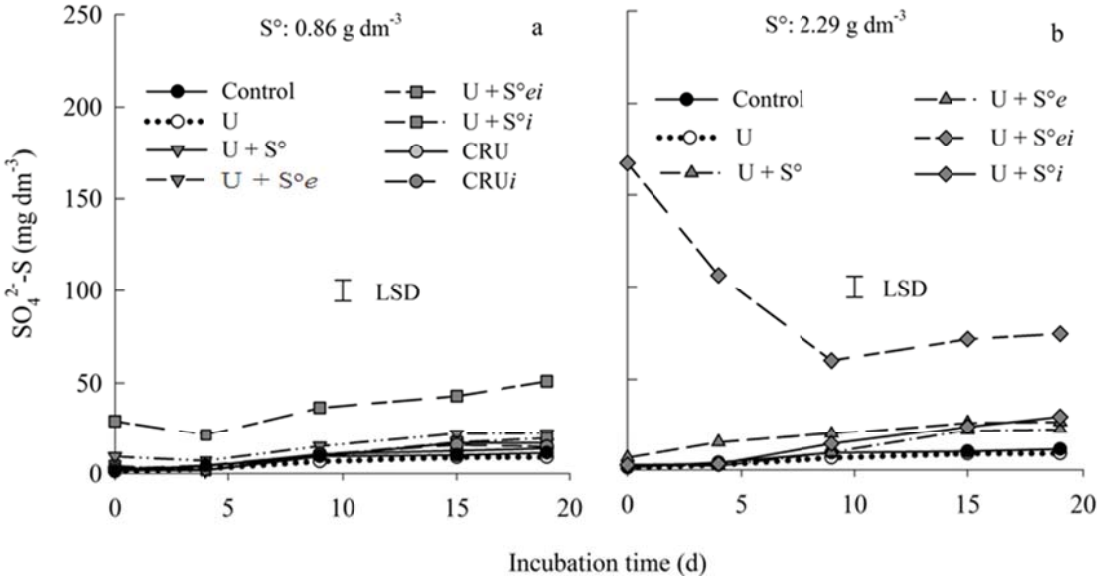

Figure 3. Concentration of SO_4^{2-}-S in soil. Treatments: control without fertilizer application; urea (U); urea + application of elemental sulfur (U + S^0); urea + early application of elemental sulfur at 12 d (U + S^0*e*); urea + early application of elemental sulfur and *A. thiooxidans* at 12 d (U + S^0*ei*); urea + early application of elemental sulfur and *A. thiooxidans* (U + S^0*i*); urea protected with elemental sulfur and polymer coating (CRU) or urea protected with elemental sulfur and polymer coating + application of *A. thiooxidans* (CRU*i*). Vertical bars indicate the least significant difference (LSD = 11.40) between treatment (Tukey test, p = 0.05)

3.4 pH

The previous S^0 application associated with the inoculation with *A. thiooxidans* affected soil pH for both S^0 doses. Indeed, the pH values decreased from 6.0 to 5.3 and 6.0 to 4.0 when S^0 was applied at doses of 0.86 and 2.29 g dm^{-3}, respectively (Figure 4). However, when urea was applied, the soil pH increased for all fertilizer treatments. The soil pH reached maximum values of 6.36 for the control, 7.49 for urea, 6.85, 7.01, 7.60, 7.68, 7.73, and 8.01 when the dose 0.86 g kg^{-1} of S^0 was used for CRU, CRU*i*, U + S^0*ei*, U + S^0*i*, U + S^0, and U + S^0*e*, respectively. When we used 2.29 g kg^{-1} of S^0, the maximum pH values were 7.01, 7.76, 7.90, and 8.15 for U + S^0*ei*, U + S^0*i*, U + S^0*e*, and U + S^0, respectively.

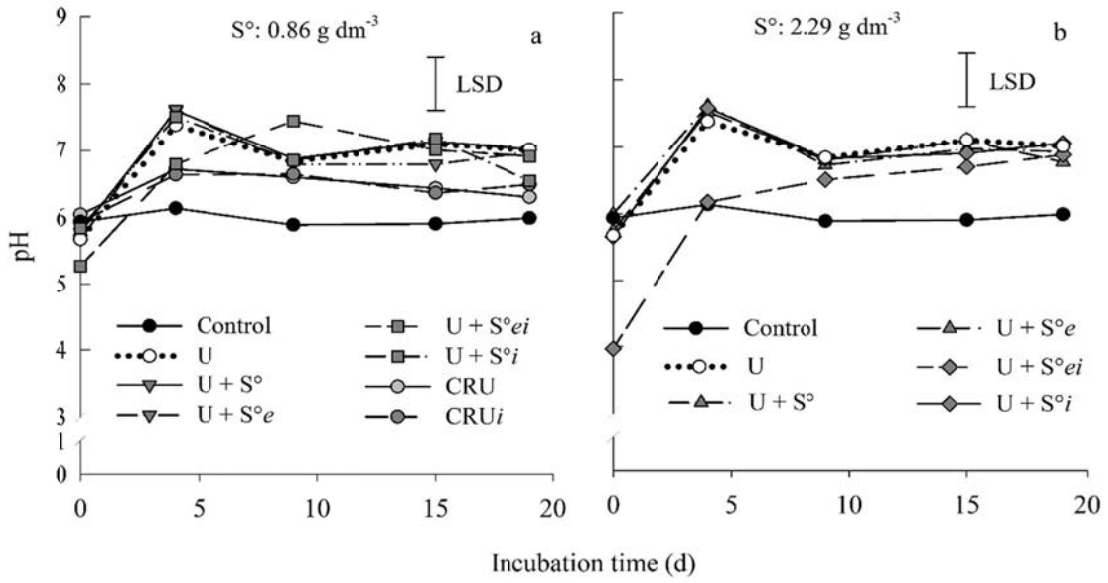

Figure 4. pH of soil: water (1:2.5) suspension. Treatments: control without fertilizer application; urea (U); urea + application of elemental sulfur (U + S^0); urea + early application of elemental sulfur at 12 d (U + S^0e); urea + early application of elemental sulfur and *A. thiooxidans* at 12 d (U + S^0ei); urea + early application of elemental sulfur and *A. thiooxidans* (U + S^0i); urea protected with elemental sulfur and polymer coating (CRU) or urea protected with elemental sulfur and polymer coating + application of *A. thiooxidans* (CRU*i*). Vertical bars indicate the least significant difference (LSD = 0.83) between treatment (Tukey test, p = 0.05)

3.5 NO_3^--N in Soil

The concentration of nitrate in soil tended to increase over incubation time; however, there was no significant difference between control and urea-based treatments. Moreover, controlled-release urea treatments had higher values of nitrate in soil from nine days after its application, especially when the S^0 dose was lower (Figure 5).

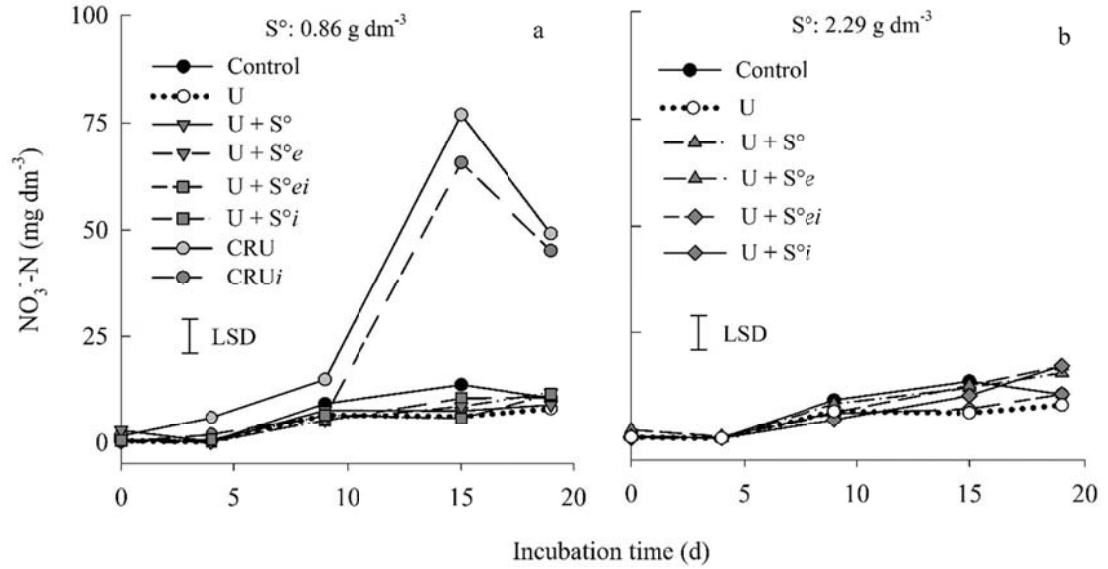

Figure 5. Concentration of NO_3^--N in soil. Treatments: control without fertilizer application; urea (U); urea + application of elemental sulfur (U + S^0); urea + early application of elemental sulfur at 12 d (U + S^0e); urea + early application of elemental sulfur and *A. thiooxidans* at 12 d (U + S^0ei); urea + early application of elemental sulfur and *A. thiooxidans* (U + S^0i); urea protected with elemental sulfur and polymer coating (CRU) or urea protected with elemental sulfur and polymer coating + application of A. thiooxidans (CRU*i*). Vertical bars indicate the least significant difference (LSD = 8.03) between treatment (Tukey test, p = 0.05)

3.6 Correlation

There was a significant positive correlation between NH_4^+ and accumulated NH_3 (0.64**), volatilization rate of NH_3 (0.59**) or pH (0.74**), but not with SO_4^{2-}-S (0.15^{ns}) (Table 3). Between pH and accumulated NH_3, the correlation was 0.55***. Furthermore, there was no correlation between SO_4^{2-} and accumulated NH_3 (0.10^{ns}).

Table 3. Coefficients for Pearson's correlation test

	NH_3 (mg/dm³/day)	NH_3 (mg/dm³-accumulated)	NH_4^+	NO_3^-	SO_4^{2-}	CE (μS/cm²)	pH
NH_3 (mg/dm³/day)	1						
NH_3 (mg/dm³-accumulated)	0.33***	1					
NH_4^+	0.59***	0.64***	1				
NO_3^-	- 0.08^{ns}	0.67***	0.25**	1			
SO_4^{2-}	- 0.09^{ns}	0.1^{ns}	0.15°	0.08^{ns}	1		
CE (μS/cm²)	0.37**	0.41***	0.65***	0.11^{ns}	0.73***	1	
pH	0.57**	0.55***	0.74***	0.19*	- 0.33***	0.22**	1

4. Discussion

The oxidation of S^0 in fact induces soil acidification, but it was overall demonstrated not to be enough to reduce ammonia volatilization from urea fertilizer. Our results showed that even with the previous application of powdered S^0 (2.29 g dm^{-3}) in soil and inoculation with *A. thiooxidans*, the time was delayed by only one day to reach 50% of the maximum NH_3 volatilization. Moreover, there were no differences between powered S^0-urea treatments on NH_3 accumulated up to day 19. It was clear that S^0 oxidation is a slow process in the soil, while the dissolution and hydrolysis of the urea granules are very fast reactions in the soil. Therefore, both processes occur without close synchrony in the soil.

We hypothesize that the kinetic of H^+ production by S^0 oxidation (Equation 1) was below the requirement to stabilize N-NH_4^+ (Equation 4), due to the fast hydrolysis of urea and the resulting N-NH_3 volatilization (de Oliveira et al., 2014) (Equations 3 and 4) associated with the low rate of S^0 oxidation in soil. Consequently, the NH_3 volatilization was reduced only up to day 9 after the application of urea, even under suitable conditions for S^0 oxidation, such as a higher S/N ratio (1.1:1), early S^0 application, and inoculation with *A. thiooxidans*. Although S^0 is a hydrophobic substance, the simple mixture with urea does not change urea granule dissolution and the dynamics of N in the soil. On the other hand, controlled-release urea, coated by S^0 and polymers, had a slowed dissolution and reduced N volatilization over time.

$$S^0 + 1.5O_2 + H_2O\ (\textit{Microorganism}) \rightarrow SO_4^{2-} + 2H^+ \quad (1)$$

$$CO(NH_2)_2 + 3H_2O\ (\textit{Urease}) \rightarrow CO_2 + 2NH_4^+ + 2OH \quad (2)$$

$$H^+ + 2OH^- \leftrightarrow H_2O \quad (3)$$

$$NH_3 + H^+ \leftrightarrow NH_4^+ \quad (4)$$

Nitrogen fertilizers such as $(NH_4)_2SO_4$ or NH_4NO_3 have less NH_3 volatilization (de Oliveira et al., 2014; Cabezas et al., 2008) because of their acid reaction in soil. On the other hand, urea hydrolysis causes the formation of CO_2, water, and NH_3 (Zavaschi et al., 2014). Such reaction tends to increase soil pH (less H^+ to convert NH_3 to $NH_4^{+)}$ around the point of its application and the losses by volatilization are intensified (Longo & José De Melo, 2005; Behera et al., 2013). Subsequently, H^+ is produced again in the soil by nitrification under oxidic conditions (Equations 5 and 6).

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + CO_2 \quad (5)$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \quad (6)$$

The previous S^0 application and inoculation with *A. thiooxidans*, especially in the higher proportion of S^0:N (1.1:1), increased the SO_4^{2-} concentration in the soil. Even though the increased concentration of SO_4^{2-}-S is an indicator of S^0 oxidation, the extractable S in the soil may underestimate the total oxidation, as our results suggest, because of both the immobilization of SO_4^{2-}-S and adsorption by soil colloids (Zhao et al., 2016). The S^0 oxidation rate in the soil is influenced by the particle size and S^0 dose (Lucheta & Lambais, 2012; López-Mosquera et al., 2015); consequently, the use of the higher dose (2.29 g dm^{-3}) powdered S^0 produced more H^+ compared to the dose of 0.86 g dm^{-3}, as was demonstrated here.

Like already demonstrated, even though inoculating S^0 with *A. thiooxidans* suspension may accelerate the S^0 oxidation in soil, the amount of produced H^+ was insufficient to counteract the urea hydrolysis reactions in terms of H^+ consumption. Moreover, we obtained low correlation coefficients between SO_4^{2-} concentrations and NH_3 volatilization rates. Interestingly, NH_4^+ and pH were positively correlated, suggesting that the effect of hydrolysis on the increase of soil pH is more predominant than the acidity due to S^0 oxidation.

From an analysis of nitrate concentration in the soil during the evaluation time, data showed that nitrification did not have important contributions to soil acidification. However, controlled-release urea treatments had more nitrate in soil compared to other fertilizer treatments, possibly because nitrification was inhibited under high NH_3 concentration and low acidity in soil (pH > 7.7) (Maharjan & Venterea, 2013; Katipoglu-Yazan et al., 2015). Hydrolysis reactions tend to be less intense with CRU because of the controlled release of urea from granules, leading to lower pH around the fertilizer application point compared to fast release urea fertilizers. The controlled-release urea has a double physical barrier that temporarily prevents the dissolution of the granule (Trenkel, 2010). Less dissolved urea in the soil solution reduces the urease activity and consequently, both the NH_4^+ concentration in soil and NH_3 volatilization are reduced.

Elemental sulfur composing controlled-release urea is less accessible for S-oxidizing microorganisms (Yasmin et al., 2007; Zhao et al., 2016) and therefore, these fertilizers have little value as a source of SO_4^{2-} in the first year of application (Boswell & Friesen, 1993; Solberg et al., 2007). In addition, our data support that inoculating *A. thiooxidans* in controlled-release urea has no influence on S^0 oxidation during the experimental time.

This study demonstrates that the chemical effects from the oxidation of S^0 in the soil are negligible in terms of stabilization of NH_4^+ when S^0 is applied in a mixture with urea or as a coating of controlled-release urea. However, applying S^0 in N fertilizers can be an inexpensive strategy to supply sulfur to plants in the medium and long term, because of its slow oxidation in soil.

5. Conclusions

Our results support that application of S^0 with urea has little effect on the chemical stability of the NH_4^+-N in the soil due to the asynchrony between the reactions of S^0 oxidation and hydrolysis of urea. Although the application of *Acidithiobacillus thiooxidans* accelerates the acidity production through S^0 oxidation, the extra H^+ was consumed by urea hydrolysis when applied in a localized manner. On the other hand, the physical barrier in controlled-release urea had less dissolved urea in soil and reduced NH_3 volatilization losses.

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Evaluation of Newly Released Cassava Varieties for Yield Performance, Reactions to Cassava Diseases and Farmers' Preference in Adjumani District of Uganda

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Abstract

Cassava viral diseases particularly cassava brown streak disease (CBSD) and cassava mosaic disease (CMD) have put pressure on cassava breeders to develop varieties that are resistant/tolerant to them. Several cassava varieties have been rolled out to farmers with the latest being NAROCASS series that are tolerant to these diseases. The yield performance of these new varieties have not been documented in some sub zones like Adjumani district that falls within a major West Nile agro-ecology of Uganda. Therefore this study sought to established yield performances of, reactions to major diseases, and farmers' preference to these newly released cassava varieties in Adjumani. Results showed significant ($P \leq 0.05$) differences among cassava varieties and experimental sites for all the parameters evaluated. Average yield performance by varieties were in the order of a local cassava—*Alifasia* (8.7 t/Ha) lowest, NAROCASS 2 (18.55 t/Ha), NASE 14 (33.97 t/Ha), NASE 19 (41.26 t/Ha), and NAROCASS 1 (41.71 t/Ha) highest. CMD foliar symptom was present at all sites on a local cassava—*Alifasia,* and on NAROCASS 1 in Ayiri parish, Ukusijoni sub-county. CBSD foliar symptoms were observed on off-types (TME 14) in the plot of NASE 14 in Miniki Parish only whereas CBSD root necrosis was observed at all sites on the local cassava—*Alifasia*, and on NASE 19 in Maaji parish, Ukusijoni sub-county. Cassava root rot disease was localised in Ukusijoni sub-county only. Farmers' preferences to these newly released cassava varieties were in the order of NASE 19 (40.96%), NAROCASS 1 (24.86%), NAROCASS 2 (15.82.28%), NASE 14 (15.54%), and a Local cassava—*Alifasia* (2.83%). Result from this study strengthens the information gap in the breeding process towards developing a cassava variety with farmer-preferred attributes, and can also inform the utilisation of these improved cassava varieties in Adjumani district.

Keywords: cassava varieties, cassava brown streak disease, cassava mosaic disease, farmers' preference, yield performance, new varieties, Adjumani

1. Introduction

Cassava (*Manihot esculenta*, Cranz) is an important root crop for food security and income generation in Sub Saharan Africa (SSA) whose potential suits the poor and marginalised farming communities (Dixon et al., 2003). The crop also has a lot of industrial applications within the SSA region and worldwide, is the third most key food source in the tropics after rice and maize and it is the staple food of at least 500 million people (Cock, 1985). Cassava has also been experimented as a potential source of bio-fuel crop in the People's Republic of China (Jansson et al., 2009). In Uganda, it's the second most important crop after banana (Ssemakula et al., 2004), and in West Nile region it is the primary crop for both food security and income generation with Arua and Yumbe investment profiles stating its importance at 53% and 38% respectively but being the highest among the crop

commodities in these districts (UIA and UNDP Report, 2019a, 2019b). Cassava has also been reported by most districts of Northern Uganda, including Adjumani as a cash crop (Mwongera et al., 2014).

In Uganda and in West Nile region cassava crop production faces a number of challenges ranging from pests and diseases (Alicai et al., 2007; Abaca et al., 2014), subsistence farming with both landraces and improved varieties under production with varying levels of cyanide content (Oloya et al., 2017), postharvest physiological deterioration (Beeching et al., 1998; Tumuhimbise et al., 2015), and unreliable marketing strategies (Roothaert & Magado, 2011) amongst others. Of all these challenges in cassava production and marketing, cassava viral diseases, particularly cassava brown streak disease (CBSD) and cassava mosaic disease (CMD) have been recognised as the greatest challenge (Kizito et al., 2005; Alicai et al., 2007). These viral diseases has encouraged a continuous cassava breeding efforts in Uganda (Kawuki et al., 2016) with several varieties being released such as the NASE (Namulonge Selection) series to the current NAROCASS (NARO Cassava) series.

During the breeding process, preliminary and advanced yields trials, in addition to multi location trials are established by the researchers from the National Programme in collaboration with the Teams from the Zonal Research Institutes to allow for participatory evaluation of yields, response to pests and diseases, and response to environmental factors by these new varieties prior to their release. In doing such, the National Agricultural Research Institutes (NARIs) such as National Crops Resources Research Institute (NaCRRI) that houses the National Roots and Tuber Crops Program considers West Nile region that is composed of 11 districts and three agro-ecological zones as a single unit for these multi-locational studies at Abi Zonal Agricultural Research and Development Institute (ZARDI). This implies that, there is an urgent need to test the yields performance and reactions to pests and diseases of these newly released cassava varieties in different locations (sub zones) of West Nile region. Therefore, the specific objectives of this study were to: i) determine the yields performance of the newly released cassava varieties within Adjumani district; ii) test the response of these newly released cassava varieties to cassava brown streak disease (CBSD) and cassava mosaic disease (CMD) in different locations within Adjumani; and iii) conduct farmers' participatory evaluation to examine their preference of these new cassava varieties in their different locations within Adjumani district.

2. Materials and Methods

2.1 Study Locations

The study was conducted in three sub counties of Ukusijoni, Itirikwa, and Dzaipi. From each sub county four Parishes were selected: Ayiri, Payaru, Kiraba, and Maaji Parishes for Ukusijoni; Kolididi, Baratuku, Mungula, and Zoka Parishes for Itirikwa; Adidi, Ajugopi, Lugwangwa, and Miniki Parishes for Dzaipi for 2018/19 season. However, experimental sites were reduced to one parish in each sub county: Ayiri parish for Ukusijoni, Mungula parish for Itirikwa and Miniki Parish for Dzaipi for 2019/2020 seasons. The decision to reduce the experimental sites from 12 to 3 parishes between the two seasons was guided by the Project Management Unit of the Project for the Restoration of Livelihoods in the Northern Region (PRELNOR) due to limited resources to cover all these sites in the second season.

2.2 Study Materials and Period

Three newly released cassava varieties comprising of NAROCASS 1, NAROCASS 2, and NASE 19; NASE 14 that has been widely adopted in the region; and a local check (*Alifasia*) were used in this study. The breeders' seeds of these three newly released cassava varieties were acquired from the National Root and Tuber Crop Programme based at the National Crops Resources Research Institute (NaCRRI), NASE 14 was sourced from Abi ZARDI and the Local cassava (*Alifasia*) was sourced from within Adjumani district. The study was run for two consecutive years of 2018/19 and 2019/20 seasons.

2.3 Experimental Design, Data Collection and Analysis

The study was established in a randomised complete block design (RCBD) across all the experimental sites. A spacing of 1 × 1 metre was used. A data collection schedules of 3, 6, 9, and 12 months after planting (MAP) were used. At 3, 6, and 9 MAP foliar data sets were collected on plant germination count per plot, plant height, cassava mosaic disease (CMD) incidence and severity, cassava brown streak disease (CBSD) incidence and severity, and farmers' preference of the plant types. CMD and CBSD severity were scored as described by Gondwe et al. (2005). At 12 MAP, a destructive sampling was used to collect the data; 10 plants were randomly selected and harvested from each experimental plot and data such as number of tuber per plant, weight of tuber per plant, lengths of longest and shortest roots, shoot biomass weight per plant and harvest index were collected. Harvest index was computed as the ratio of the weight of the roots to the total biomass weight (weight of roots plus stool). Cassava brown streak disease (CBSD) root necrosis assessment was also conducted as previously

described by Abaca et al. (2012a, 2012b) to determine the varietal response to CBSD in Adjumani district. Farmers' preference to these newly released varieties was assessed through lining up. Briefly, after harvesting the 10 plants of each plots, the fresh roots and shoot (planting materials) were bulked, farmers were asked to taste the fresh roots and observed the plant characteristics, and lined up in close proximity their variety of choice. The mean of the collected data was summarised in an excel file and subjected to PAST3 and R software for statistical analyses based on sub-counties, parishes and varieties as presented in the result section below.

3. Results

3.1 Yields Performance of Newly Released Cassava Varieties

Cassava yields and yield related parameters that were evaluated at 12 MAP varied significantly ($p \leq 0.05$) by experimental sites, cassava varieties and seasons. Cassava yields and yield parameters variations across experimental sites and season are summarised in Table 1. Cassava yield rankings by parishes for each sub country are also summarised in Table 1. Briefly, for Ukusijoni sub-county the cassava yield was highest in Ayiri (46.22 t/Ha), Kiraba (38.03 T/Ha), Maaji (37.75 t/Ha) and Payaru (37.13 t/Ha) parishes in that order; for Itirikwa sub-county it was in the order of Baratuku (41.08 t/Ha), Mungula (37.98 t/Ha), Kolodidi (37.22 t/Ha) and Zoka (19.93 t/Ha) parishes; and for Dzaipi it followed Ajugopi (29.77 t/Ha), Loguangwa (25.68 t/Ha), Miniki (22.23 t/Ha) and Adidi (19.93 t/Ha) parishes, all for 2018/19 season. Overall, the highest number of fresh roots per plants was recorded in Kiraba, Ajugopi, and Miniki parishes in 2018/19 season; highest weight of root per plant was recorded in Ayiri parish in 2018/19 season while the lowest was recorded in Zoka parish for the same season; and the highest fresh root yields was recorded in Ayiri parish while the lowest was recorded in Zoka parish all for 2018/19 season (Table 1). Across locations and seasons irrespective of the varieties evaluated, cassava yields ranged between 21.22 and 36.93 t/Ha in Itirikwa and Ukusijoni respectively, with a mean of 29.18t/Ha (Table 2). Fresh root yields decreased from 2018/19 to 2019/20 seasons for Ukusijoni and Itirikwa sub counties but increased for the same period in Dzaipi sub-county (Table 2). Fresh root weight per plant ranged from 3.04kg to 5.02kg both values were recorded in Dzaipi for 2018/19 and 2019/20 seasons respectively with a mean of 4.69kg (Table 2). Average length of roots ranged between 47.00 cm to 68.83 cm all in Dzaipi sub-county with a mean of 57.08 cm. By cassava variety, yields ranged from 5.46t/Ha to 44.38t/Ha for the local cassava—*Alifasia* and NASE 19 respectively, with a mean yields by variety stood at 28.68t/Ha (Table 3). Average fresh root weight per plant ranged from 1.95kg to 7.1kg for the local cassava—*Alifasia* and NASE 19 respectively with a mean of 4.01kg (Table 3).

Table 1. Cassava yields and yields parameters variation across experimental sites and season

Season	Sub-county	Parish	Av. No of fresh roots/plant	Av. Wt of fresh roots/plant	Av. Fresh roots Yields (t/Ha)	Av. Longest roots length (cm)	Av. Shortest roots length (cm)	Yield Rankings by parishes
2018/19	Ukusijoni	Maaji	8	4.7	37.75	47.72	11.78	5
		Ayiri	9	**5.78**	**46.22**	55.78	**14.79**	**1**
		Kiraba	**10**	4.75	38.03	47.89	12.15	3
		Payaru	9	4.64	37.13	53.36	13.14	7
	Itirikwa	Kolididi	9	4.65	37.2	55.23	13.47	6
		Baratuku	9	5.13	41.08	52.52	13.61	2
		Zoka	7	2.42*	19.39*	48.63	14.01	12
		Mungula	8	4.75	37.98	54.79	14.08	4
	Dzaipi	Adidi	8	2.49	19.93	42.53*	11.86	11
		Loguangwa	7	3.21	25.68	45.67	12.42	9
		Ajugopi	**10**	3.72	29.77	52.37	10.68	8
		Miniki	**10**	2.78	22.23	47.43	11.01	10
2019/20	Ukusijoni	Ayiri	8	4.92	36.93	56.37	11.13	1
	Itirikwa	Mungula	8	4.13	21.22	66.32	9.23*	3
	Dzaipi	Miniki	7	5.02	29.39	**68.83**	9.59	2
Overall mean			**8**	**4.33**	**32.90**	**53.78**	**12.41**	

Note. Bold values: highest values; *: lowest value.

Table 2. Cassava yields and yield related parameters observed from three sub counties of Adjumani

Yield parameters	Sub Counties						Overall mean
	Ukusijoni		Itirikwa		Dzaipi		
	2018/19	2019/20	2018/19	2019/20	2018/19	2019/20	
Av. No of fresh roots/plant	**9**	8	8	8	**9**	7	**8**
Av. Wt of fresh roots/plant	4.97	4.92	4.24	4.13	3.05	**5.02**	**4.39**
Av. Fresh root yields (t/Ha)	**39.78**	36.93	33.91	21.22	24.40	29.39	**30.94**
Av. Longest roots (cm)	51.19	56.37	52.79	66.32	47.00	**68.83**	**57.08**
Av. Shortest roots (cm)	12.97	11.13	13.79	**9.23**	11.49	9.59	**11.37**

Note. Av: average; No: number; Wt: weight; Bold values: highest values for the different parameters.

Table 3. Cassava yields and yield related parameters from the newly released cassava varieties in Adjumani;

Yield parameters	Cassava Varieties										Overall Mean
	LOCAL		NASE 14		NAROCASS 1		NAROCASS 2		NASE 19		
	2018/19	2019/20	2018/19	2019/20	2018/19	2019/20	2018/19	2019/20	2018/19	2019/20	
Av. No of fresh roots/plant	6	3	**10***	8	9	9	8	7	8	**10***	**7.00**
Av. Wt of fresh roots/plant	2.8	1.95	3.85	5.2	5.8	5.79	2.77	2.96	4.99	**7.1***	**4.01**
Av. Fresh root yields (t/Ha)	11.94	5.46	31.06	36.87	**46.42***	41.71	22.13	14.96	38.13	44.38	**27.40**
Av. Longest roots (cm)	43.26	37.56	49.51	68.43	58.2	65.93	42.38	62.24	55.57	**81.4***	**53.68**
Av. Shortest roots (cm)	11.94	10.81	11.44	10.78	13.89	**8.73***	11.84	8.85	13.53	11.24	**11.59**

Note. Av: average; No: number; Wt: weight; Bold values: highest values for the different parameters.

3.2 Reactions to Cassava Diseases by These New Cassava Varieties Across the Study Sites

The major cassava diseases that these new cassava varieties reacted to were cassava mosaic disease (CMD), cassava brown streak disease (CBSD), and cassava root rot disease (CRRD) but their reactions varied across locations and varieties (Table 4). CMD was present at all sites on the local cassava—*Alifasia* with the disease incidence of 100% and severity score of 4 for both 2018/19 and 2019/20 seasons, and only on NAROCASS 1 (23% and score of 3) during the 2019/20 season for the first 6 months and later recovered in Ayiri parish, Ukusijoni sub county (Table 4).

CBSD root necrosis was observed in all the sites of Ukusijoni and Dzaipi sub counties and only in Kolididi parish of Itirikwa subcounty on the local cassava—*Alifasia* (84% with a score of 4; 90% with a score of 4) for the 2018/19 and 2019/20 seasons respectively (Table 4), and on NASE 19 (12.3% with a score of 3) only in Maaji parish of Ukusijoni subcounty (Figure 1) for 2018/19 season. CBSD wasn't observed across all sites except on one plot of NASE 14 in Miniki parish for the 2019/20 season. However, on close examination of this plot, we discovered that the three plants with visible CBSD foliar symptoms were off-types of TME 14, a cassava variety that had succumb to CBSD severely.

CRRD was present on NAROCASS 1 (23% with a score of 3, and 33% with a score of 3) for 2018/19 and 2019/20 seasons and on NASE 19 (13% with a score of 2) in 2018/19 season but restricted to Maaji and Ayiri parishes of Ukusijoni sub-county (Table 4).

Figure 1. CBSD root necrosis on NASE 19 (A) in Maaji Parish and on Local—*Alifasia* (B) in Ayiri Parish, Ukusijoni sub-county

3.3 Farmers' Preference to Cassava Varieties Across Study Sites

Farmers' preferences to these newly released varieties varied by locations, gender and seasons (Table 5). For 2018/19 season, farmers preferred NASE 19 (42.29%), NAROCASS 1 (26.88%), NASE 14 (16.6%), NAROCASS 2 (10.28%) and Local (0.04%) in that order whereas for 2019/20 season the order of preference changed to NASE 19 (38.78%), NAROCASS 2 (29.59%), NAROCASS 1 (18.37%), NASE 14 (13.27%) and local (0%). Overall, NASE 19 was a number one choice in all the experimental sites except in Ayiri parish during 2019/20 seasons. Farmers supported their preferences to these new varieties by providing several reasons: yields, planting materials characteristics, tolerance to CBSD, dry matter content, root size and shape, plant vigour to smoothen weeds, early maturity, experience in growing that particular variety, sweetness, susceptibility to CMD, ready market for the stems amongst others.

Table 4. Incidence and Severity of major cassava diseases identified during this study in Adjumani district

Cassava Variety	2018/19 Season								2019/20 Season							
	CBSD-fi (%)	CBSD-fs Score	CBSD-ri (%)	CBSD-rs Score	CMD-i (%)	CMD-s Score	CRRD-i (%)	CRRD-s Score	CBSD-fi (%)	CBSD-fs Score	CBSD-ri (%)	CBSD-rs Score	CMD-i (%)	CMD-s Score	CRRD-i (%)	CRRD-s Score
ALFASIA	0	1	84	4	100	4	0	1	0	1	90	4	100	4	0	1
NASE 14	0	1	0	1	0	1	0	1	12	3	0	1	0	1	0	1
NAROCASS 1	0	1	0	1	0	1	23	3	0	1	0	1	23	3	33	3
NAROCASS 2	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1
NASE 19	0	1	12.3	3	14	2	13	2	0	1	0	1	0	1	0	1

Note. . CBSD-fi: cassava brown streak disease foliar incidence; CBSD-fs: cassava brown streak disease foliar severity; CBSD-ri: Cassava brown streak disease root incidence; CBSD-rs: cassava brown streak disease root severity; CMD-i: cassava mosaic disease incidence; CMD-s: cassava mosaic disease severity; CRRD-i: cassava root rot disease incidence; and CRRD-s: cassava root rot disease severity.

Table 5. Summary of farmers' preferences to the newly released cassava varieties in Adjumani district

Year	Sub-county	Parish	Preference to cassava varieties by farmers									
			NAROCASS 1		NAROCASS 2		NASE 19		NASE 14		LOCALS	
			F	M	F	M	F	M	M	F	F	M
2018/19	Ukusijoni	Maaji	3	1	4	3	4	3	0	0	0	1
		Ayiri	3	3	2	2	6	3	0	0	0	0
		Kiraba	6	2	2	1	4	5	0	0	0	0
		Payaru	4	1	4	2	4	3	4	4	0	0
		Sub total	**16**	**06**	**12**	**08**	**28**	**14**	**04**	**04**	**0**	**1**
	Itirikwa	Kolididi	3	4	0	0	9	2	5	0	0	0
		Baratuku	3	3	0	0	2	2	2	2	0	1
		Zoka	1	3	2	2	4	4	4	1	1	0
		Mungula	1	2	0	0	3	3	2	1	1	1
		Sub total	**8**	**12**	**2**	**2**	**18**	**11**	**13**	**04**	**2**	**2**
	Dzaipi	Adidi	4	4	0	0	3	7	3	2	2	1
		Logwangwa	3	3	0	1	2	4	2	1	0	1
		Ajugopi	4	2	0	1	6	5	3	1	0	1
		Miniki	3	4	1	0	4	5	2	3	0	0
		Sub total	**14**	**12**	**1**	**1**	**15**	**21**	**10**	**07**	**2**	**3**
	2018/19 Sub Total		**38**	**32**	**15**	**11**	**61**	**46**	**27**	**15**	**4**	**6**
			70		**26**		**107**		**42**		**10**	
2018/19 Preference Ranking			**2**		**4**		**1**		**3**		**5**	
2019/20	Ukusijoni	Ayiri	02	01	08	06	04	02	03	02	0	0
	Itirikwa	Mungula	04	03	00	01	08	06	02	03	0	0
	Dzaipi	Miniki	04	04	06	08	10	08	02	01	0	0
2019/20 Sub total			**10**	**08**	**14**	**15**	**22**	**16**	**07**	**06**	**0**	**0**
			18		**29**		**38**		**13**		**0**	
2019/20 Preference Ranking			**3**		**2**		**1**		**4**		**5**	
Grand Total			**48**	**40**	**29**	**27**	**83**	**62**	**34**	**21**	**04**	**06**
			88		**56**		**145**		**55**		**10**	
Overal Preference Ranking			**2**		**3**		**1**		**4**		**5**	

4. Discussions

The main objectives of this study were to determine yields performance of newly released cassava varieties in West Nile region of Uganda, test the reactions of these new cassava varieties to major cassava diseases particularly CMD and CBSD, and conduct participatory evaluation by farmers to assess their preference to these new cassava varieties. This study shares similar finding with that of Abaca et al. (2014) on the variation of yields among newly released cassava varieties across West Nile region that ranged from 22.7 to 53.0 t/ha in 2013 seasons. Yield variations in cassava have been observed in several countries with different factors being advanced for these variations (Nebiyu, 2006; Ntawuruhunga & Dixon, 2010; Suja et al., 2010; Gbadegesin et al., 2010). Ntawuruhunga and Dixon (2010) observed yield variation amongst cassava genotypes in Uganda and pointed that these variations could be attributed to both genetic and environmental factors. The same study also pointed out that root number, length and size make up a significant component of yields in cassava. This current study finds a positive pair wise correlation between root yields and root length, and between root yields and root numbers. Nebiyu (2006) worked on Ethiopian cassava varieties and partitioned the cause of these variations into environmental and genetic factors for the different yield related parameters. Suja et al. (2010) showed that tuberous root dry matter and total dry matter production, crop growth rate, tuberous root bulking rate and harvest index, number of tuberous roots, mean weight of tuberous roots and nutrient uptake showed significant positive correlations with tuberous root yield in Kerala, India. The study of Gbadegesin et al. (2010) in Nigeria indicated that cassava shoot biomass and yields parameters are strongly linked soil properties using Pearson's product Moment Correlation and Multiple regression analytical techniques. Therefore the low yields in Zoka parish can be attributed to both environmental and soil properties, particularly the thick vegetation covers that were cleared from the study site prior to establishing the trial. Therefore, in this current study we can suggest that variations in

yields and yield parameters across sites could be attributed to environmental and soil related factors whereas variations amongst cassava varieties could be attributed to genetic factors.

The presence of CMD on NAROCASS 1 only for the first 6 months after plantings shows its potential to recover from the cassava mosaic virus infection. Thresh et al. (1998) made a similar observation that cassava mosaic virus that causes CMD sometimes is not fully systemic within infected plants and the infected plants are able to reverse or recover from such infections. However, it should be noted here that, the ability to recover from CMD infection is mainly associated with the white flies infection compared to the cutting infection. Therefore, it is important for small scale farmers to start with clean planting materials. The presence of CBSD root symptoms without foliar symptoms on Local cassava—*Alfasia* in all sites, and on NASE 19 in Maaji parish, confirms the finding of Abaca et al. (2012b) that different cassava varieties respond to CBSD differently, hence it is not conclusive enough to make decision based on foliar symptoms alone during the seed crop inspection in the fields. The restriction of CRRD to only Ukusijoni subcounty could suggest environmental and soil related variations that occurred between the experimental sites. Akrofi et al. (2018) through their work on CRRD in Ghana reported that the pathogens that cause CRRD (*Botryodiplodia theobromae*; *Fusarium solani*; *Fusarium oxysporum*; *Fusarium semitectum*; and *Sclerotium rolfsii*) are soil borne and thus spread through soil, and that CRRD incidence increases during the rainy season. Additional factors such as cultivation of susceptible cassava varieties, delayed harvesting, cultivating cassava in waterlogged soils, and high weed density were also suggested by their same work in Ghana. Similarly, the work of Makambila (1994) in the Republic of Congo had indicated also indicated high humidity near saturation and a temperature range of 24-28 ^{o}C was required for CRRD to develop. Therefore, examining our study sites, Ukusijoni subcounty (Maaji and Ayiri parishes) where CRRD was restricted had a very high amount of rainfall (same as relative humidity) and would occasionally flood which supports the finding reported in Ghana and Republic of Congo above. The reasons presented by farmers in this present study during participatory evaluation of these newly released cassava varieties confirm the reasons presented by Abele et al. (2008) in Uganda, Kavia et al. (2007) in Tanzania, Udensi et al. (2011) and Nwakor et al. (2011) in Nigeria for factors promoting adoption of new cassava varieties in these countries. The increase in the preference of NAROCASS 2 from 10.28% in 2018/19 season to 29.59% in 2019/20 season agrees with the finding of Kavia et al. (2007) that lack of information on a particular technology and farmers experience about a technology affects its adoption. Growing and observing the performance of NAROCASS 2 in the first season as a new variety in Adjumani could have increased farmers' awareness about it in the second season, thus, the increment in the percentage of its preference. Furthermore, factors such as age, marital status, educational levels, gender, farm size, economics of production of a technology, land ownership, complexity of a technology amongst others have been described as key factors in the adoption a technology by different Authors (Kavia et al., 2007; Udensi et al., 2011; Nwakor et al., 2011; Mwangi & Kariuki, 2015).

4. Conclusion and Recommendations

We have demonstrated in this research that cassava can be grown in any part of Adjumani district including Miniki Parish in Dzaipi sub-county where cassava production has been lowest although variations in yields and yields related parameters from the newly released cassava varieties were great within the district. Similarly, response to cassava diseases varied among locations and varieties with cassava root rot disease being restricted in Ukusijoni sub-county only. Despite the variations in different parameters, farmers were able to make informed decisions and selected NASE 19 and NAROCASS 1 as their best cassava varieties.

From the results and discussions section above, we can therefore recommend the following from this study:

(i) Farmers' adoption of a particular cassava varieties results from a combination of several factors that require adequate time for probing. This explains why NAROCASS 2 wasn't selected by farmers despite an increase in its yields in the second season.

(ii) The District Crop Inspectors should not rely upon the CBSD foliar symptoms as the disease may not show foliar symptom but will show root necrosis.

(iii) Itirikwa sub-county should be considered as a cassava seed multiplication site for Adjumani district. This is because although it has a low fresh root yields as shown in Zoka, it supports very high shoot biomass that is good for seed multiplication. Additionally, Itirikwa sub-county has low rate of cassava stem destruction by stray animals during dry season.

(iv) NASE 19 and NAROCASS 1 being the most preferred cassava varieties in Adjumani district, therefore, any agribusiness innovation on cassava in the district can be sought alongside these two varieties.

(v) CBSD and CMD are present whenever cassava is being grown and therefore farmers should be encouraged and supported to cultivate the improved cassava varieties to safeguard their food security and income status.

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Desmodium tortuosum, *Euphorbia heterophylla* and *Moringa oleifera* Effect on Local Rabbit Does Milk Production and Pups' Performances

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Abstract

Desmodium tortuosum and *Euphorbia heterophylla* are fields' weeds. *Moringa oleifera* plant is adapted to several agroecological zones and has many food and medicinal virtues. This work assessed these three plants potential to induce milk production. Thus, 96 primiparous local breed rabbit does, 10 months old, with an average 2983.6±212.4 g weight were used. They were grouped into 4 blocks containing 24 animals each. Then, one diet among 4 diets was randomly assigned to each group. *Panicum maximum* as fodder was mixed with a commercial pellet rabbit feed, the control (*Pan*). Then, this control diet was supplemented with *Desmodium tortuosum* (*Des*), *Euphorbia heterophylla* (*Eup*) and *Moringa oleifera* (*Mor*) in pellet partial substitution. The parameters monitored were the litter size, the pups' average daily weight gain, the does' weights before and during gestation, and after farrowing. Likewise, the milk production at peak lactation was evaluated. As a result, compare to *Pan*, *Des*, and *Eup* diets improved the total rabbit pups' number from 96 to 112, and it represented a 16.67% gain. But *Mor* diet reduced *Pan* diet performance to 76 newborn rabbits, it was a 20.83% loss. Moreover, *Des*, *Eup*, and *Mor* diets induced an improvement in the milk quantity at peak lactation. In this order, these improvements were +15.51, +27.74, and +19.98%, respectively, compared to *Pan* diet which produced 109.6 g. In conclusion, *Desmodium tortuosum* and *Euphorbia heterophylla* could be used as green forages to improve milk production in local rabbit does breeding.

Keywords: *Desmodium tortuosum*, *Euphorbia heterophylla*, *Moringa oleifera*, rabbit does, litter size, milk production

1. Introduction

In African flora, several plants are used for therapeutic and food purposes. Accordingly, Yapo et al. (2012) demonstrated *Parkia biglobosa* leaves aqueous extract immunostimulant activity in rabbits. Again, Kouakou et al. (2015, 2016) came to the conclusion that *Euphorbia heterophylla*, and *Ipomea batatas* leaves and stems can be used as supplements in rabbit and guinea pig diets. These leaves induce a good organic matter digestibility while promoting a significant reduction in production costs. Given the resulting animal products' good nutritional quality in terms of their polyunsaturated fatty acids content, HDL cholesterol contents, and high antioxidant power, these species are also recognized for their dietary and medicinal properties (Kouakou et al., 2015, 2016; Kouassi et al., 2017). Moreover, plants with galactogenic effects are also medicinal plants that are widely used in rural areas where breast milk remains the main route for babies' breastfeeding. For example, Sepehri et al. (1992) observed that crude and partially purified extract injection of coma (*Sideroxylon celastrinum*), flax (*Linum usitatissimum*), and cotton (*Gossypium hirsutum*) intravenously to sheep induced the prolactin secretion. Codjia et al. (2001), Deleke-Koko et al. (2011), and Akouedegni et al. (2012) identified steroidal constituents, terpenes, cardiotonic derivatives, saponins, and tannins presence in *Adansonia digitata* leaves. These metabolites would contribute to its lactogenic effect.

Herein work hypothesis was that "*Desmodium tortuosum*, *Euphorbia heterophylla* and *Moringa oleifera* green forages would act on local rabbit does zootechnical and their litters performance". Thus, the main objective was to evaluate these green forages galactogenic effect served with a commercial mixed granulated feed for rabbits. The specific objectives were the local rabbit does dry matter digestibility evaluation, the milk production at the lactation peak, and the young rabbits' average daily weight gain.

2. Materials and Methods

The experiment was carried out from April to June 2019, at the experimental farm of the National Polytechnic Institute Felix Houphouët Boigny (INP-HB). Specifically, the essay was established at the laboratory of Zootechnics and Animals Productions, at Yamoussoukro (6.5° N; 5.2° W) in Côte d'Ivoire (Ivory Coast). During the test, the average temperature was 29.6±1.2 °C, and the relative humidity fluctuated between 76.7±2.3% and 79.0±4.2%. Finally, this period's average monthly rainfall was 111.6±15 mm.

2.1 Animals and Housing

The animals were bred and slaughtered in accordance with the regulations for the care and use of research animals, according to European Directive 86/6096. The National authorization to experiment on live animals' number 3502 was issued to Maryline Kouba by the French Ministry of Agriculture. This regulation is accepted and applied in Côte d'Ivoire. So, ninety-six (96) multiparous local breed rabbits (*Oryctolagus cuniculus*), with an average weight of 2983.6±212.4 g and 10 months old were used. Four groups of 24 rabbits does each were set. The rabbits were randomly distributed to individual maternity cages. The cages had 108 dm^3 volume (90cmx40cmx30cm, respectively for length, width and height) standing wire mesh, in a covered, and very well-ventilated rearing building. Each group was subjected to one of the four diets studied throughout the experiment. Thus, eight males with an average 2984.6±213.4 g weight were used for breeding.

2.2 Feed Ingredients Chemical Analyzes

The diet ingredients (pellet (FACI, Abidjan, Côte d'Ivoire) and green fodder) to be tested have been analyzed. These analyses consisted of dry matter (DM), ash, fat, protein, and crude fiber (CF) determinations. Total fiber contents were determined using the Fibertec System hot extractor. In addition, all the analyzes were carried out according to AOAC (1990). In addition, the ingredients, their total carbohydrate and their associated metabolizable energies (M.E.) were determined according to FAO (2003) approach (Table 1).

Table 1. Chemical composition of experimental diets' ingredients

Composition (%DM)	*Desmodium tortuosum*	*Euphorbia heterophylla*	Pellet	*Moringa oleifera*	*Panicum maximum*
Dry matter	22.9	17.4	90	13.3	25.8
Organic matter	89.3	91.8	90.1	88.6	90.7
Ash	10.7	8.2	9.6	11.4	9.3
Crude fiber	24.9	17.1	14.6	24.0	17.4
Protein	19.5	16.5	16	22.7	10.2
Fat	2.4	2.3	3.3	2.8	2.0
Total carbohydrate	67.4	73.0	71.1	63.1	78.5
M.E. (kcal/kg DM)	3082.86	3201.21	3204.88	3040.91	3218.73

Note. M.E.: Metabolizable Energy.

Total Carbohydrate (Tot_Carb) = 100% (Protein + Fat + Water + Ash) (FAO, 2003).

M.E. (kcal/kg DM) = [2.44 × Protein (%DM) + 8.37 × Fat (%DM) + 3.57 × Tot_Carb (%DM)] × 10 (FAO, 2003).

2.3 Experiments Procedure

The tests lasted 81 days divided into 3 periods, including adaptation to the experimental environment for 23 days, gestation which lasts 30 days, and the young rabbits suckling lasted 28 days. The rabbit does mating was done after the adaptation period. Then, the diets were made by one control and three experimental diets. The control was composed of *Panicum maximum* Orstom G23 variety and some mixed rabbit pellets bought from FACI© (*Pan*). Next, the experimental diets were made of a control diet associated with *Euphorbia heterophylla* (*Eup*), *Desmodium tortuosum* (*Des*), and *Moringa oleifera* (*Mor*) (Table 2). All ingredients were collected in the wild, under natural conditions. The herbs such as *Euphorbia heterophylla* and *Desmodium tortuosum* were cut at 5 cm

upper to the ground. But, *Moringa oleifera* fresh branches were cut at different positions depending on their position on the tree. Every day, the ingredients were weighed and distributed in order to evaluate the food intake. The minimum required quantities per animal and per day have been increased so that they become ad libitum. The collected green fodder was cleaned with potable water containing 2% bleach, pre-dried under the sun for half a day. Then, they were served the next day at 8 a.m. Thus, the parasite pressure was reduced a little bit. Unfortunately, this pre-dried process was not possible with *Moringa oleifera*. In fact, *Moringa oleifera* leaflets were coming off from the branches during the pre-drying process. So, *Moringa oleifera* forage was harvested, cleaned, and served daily. Finally, the pellets were given in the afternoon (5 PM).

Table 2. Diets, ingredients, distributed amounts, ingredient incorporation and diet characteristics

Diet	Ingredients	Distributed amounts	Incorporation rate (%)	Fat (%DM)	Protein (%DM)	M.E. (kcal/kg DM)
Pan	*Panicum*	116.14	51.98	2.63	12.99	3212.58
	*Pellets	107.30	48.02			
Des	*Panicum*	232.29	57.11	2.32	13.66	3179.41
	*Pellets	61.34	15.08			
	Desmodium	113.10	27.81			
Eup	*Panicum*	116.14	19.87	2.30	15.22	3205.70
	*Pellets	30.17	5.16			
	Euphorbia	438.22	74.97			
Mor	*Panicum*	232.29	51.69	2.48	15.30	3157.50
	*Pellets	64.20	14.29			
	Moringa	152.86	34.02			

Note. M.E.: Metabolizable Energy.

Total Carbohydrate (Tot_Carb) = 100% (Protein + Fat + Water + Ash) (FAO, 2003).

M.E. (kcal/kg DM) = [2.44 × Protein (%DM) + 8.37 × Fat (%DM) + 3.57 × Tot_Carb (%DM)] × 10 (FAO, 2003).

* These FACI pellets were made of corn, corn bran, coconut meal, cottonseed meal, molasses, and limestone premix.

2.4 Feed Intake and Apparent Digestive Utilization Coefficient

The refusals were collected daily, weighed, and placed in an oven at 70 °C to estimate their dry matter content (DM). Thus, the voluntary dry matter (DM) amount ingestion could be assessed. Using a scale (Haier, Qingdao, China, model-332L, maximum 5000 g, precision 1 g), the rabbit does' body weights were recorded weekly throughout the experimental period. Similarly, another small scale (Kern, Balingen, Germany, model WLPC6S, maximum 3000 g, precision 0.01 g) was used to weigh the young rabbits at birth and weekly until age 28 days. So, these weight records helped to assess the rabbit does and young rabbits' average daily weight gain (ADWG) during the lactation. Also, each rabbit doe's droppings were collected and recorded daily after the third week of gestation (22^{nd} to 26^{th} days) and their DM content was evaluated in order to determine the apparent digestive utilization coefficient (1). In detail, during 5 days, at 8 A.M, 5 rabbit does were randomly selected per diet. Thereafter, their droppings were collected. Just after collection, they were weighed fresh, then taken to an oven and dried at 105 °C for 24 hours, separately according to each rabbit doe. After the drying, the feces of each rabbit doe were weighed. So, an average apparent digestive utilization coefficient was computed per diet, based on the 5 data.

$$DUC\ (\%) = [(\text{Feed intake} - \text{Feces})/\text{Feed intake}] \times 100 \quad (1)$$

2.5 Milk Production

Pups rabbits' numbers were equalized to six per rabbit does within each group immediately after birth by adding or removing newborn rabbits. The milk production was estimated during the entire breastfeeding period. The day before measurement days, each rabbit does was separated from her young rabbits from 6 P.M. to 7 A.M. To begin, each rabbit does was weighed. Then, the newborns were put together with the rabbit does for 15 minutes. To end, each rabbit does was weighed again after the breastfeeding. So, the change in weight decrease before and after breastfeeding corresponded to the daily milk production (Lebas, 1971; Zerrouki et al., 2012).

2.6 Statistical Analysis

Data were analyzed by the one-way analysis of variance (ANOVA) option of the generalized linear model (GLM) of XLSTAT version 2014, 2014.5.03 (Copyright© 1995-2014 Addinsoft Sarl, Paris, France) as 2 treatments with diet as main effects. The statistical model used was $Y_{ik} = \mu + D_i + R_i + \gamma_{ik}$, where, Y_{ik} = response variables from each individual replication or pen, μ = the overall mean; D_i = the effect of diet; R_i = the inter-experimental unit (replications) error term; and γ_{ik} = the intra-experimental unit error term (Koné et al., 2020). Least significant difference comparisons were made between treatment means for main effects when there was a significant F value. Significance implies $P < 0.05$, unless stated otherwise.

3. Results and Discussion

3.1 Diets Digestibility

The diet digestibility is presented in Table 3. The dry matter (DM) voluntary daily intake means by Rabbit does subjected to *Pan*, *Des*, *Eup*, and *Mor* diets were significantly different. Indeed, rabbits does under *Des* and *Pan* diets were significantly higher than those of *Eup* and *Mor* diets. Numerically, *Des* and *Pan* diets led to 122.2 g per day on average. Based on dry matter ingestion, among *D. tortuosum*, *E. heterophylla*, *M. oleifera*, and *P. maximum* experimental diets, *E. heterophylla* was the most ingested forage for 68.3 and 70.1 g/d, respectively during gestation and lactation. While, *M. oleifera* was the less ingested for 12.3 and 24.3 g/d, respectively during gestation and lactation. Looking at *P. maximum* in different diets, its highest intake level was observed with *Des* diet for 46.5 and 51.7 g/d during the gestation and lactation, respectively. On the contrary, the lowest level was obtained with *Eup*, for 18.2 and 19.9 g/d, in the same physiological period order. Singularly, *P. maximum* ingestion levels in *Pan* and *Mor* diets were not significantly different; 34 and 32.7 g/d, respectively, during gestation ($P > 0.05$).

A careful look at the voluntary daily intake of metabolizable energy shows an increase from gestation to lactation. So, from 391.5 to 425.98 kcal per day, *Pan* diet energy intake increased by 8.81%. Similarly, *Des* diet energy intake increased by 7.12%, from 390.44 to 418.28 kcal per day. This increase in energy intakes from gestation to lactation was reported by Fernández-Carmona et al. (2003). Again, metabolizable intake increased with *Eup* diet by 9.94% by moving from 341.74 to 375.71 kcal per day. Finally, increasing from 327.96 to 391.4 kcal per day, the energy consumption in *Mor* diet rose up by 19.34%.

The observed differences in daily dry matter intake during the lactation period were linked to the variations in diets' pellet quantities. Its quantities derived from calculus which aimed to provide iso-protein and iso-energetic diets. In all diets, the pellets were fully consumed, and the animals compensated the lacks with *Panicum maximum* and the test forage. So, *Des* diet got the best ingestion level for 131.2±8.3 g DM, followed by *Pan* diet for 122.0±9.1, *Eup* diet for 117.1±7.6 g MS, and finally by *Mor* diet for 102.8±9.2 g MS. This *Mor* diet lowest ingestion level could be explained by *Moringa oleifera* inappetence because the plant was harvested and distributed on the same day, without any pre-drying.

Indeed, the feed presentation is an important factor modulating rabbits' ingestion. According to Gidenne and Lebas (2005), in free choice, rabbits prefer pellet feed for 97%. In fact, pellet feeds are compact and dry (Kpodékon et al., 2009; Lebas, 2007). The pellet benefits from a fine grain size of its particles size due to the grinding and nutritional values. For example, pellets are made of minerals, vitamins, and amino acids of plant or animal origin, dried coconut, and cotton cake which are used as protein sources. Thus, when rabbits have a choice, they derive greater profit from it (Kouakou et al., 2016). Indeed, the rabbit digests fine particles better than coarse particles (Gidenne & Lebas, 2005). During digestion transit through the large intestine muscle, rabbits' practice fine particles retention selective fluids through colon walls which separates particles according to their size (Sakaguchi, 2003). Then, the pellet small amount in the *Eup* diet will lead to a large amount of *Euphorbia heterophylla* ingestion, to fill its energy need. However, because of the stomach congestion phenomenon, the fresh forage ingestion capacity is fundamentally limited (Kouakou et al., 2016).

All diets induced an increase in daily weight gain for rabbit-does (Table 3). This increase was significantly elevated ($P < 0.05$) with rabbit does fed on *Pan* diet based on *Panicum maximum* and rabbit mixed concentrate pellets for 15.7±6.1 g and *Desmodium tortuosum* (Des) diet for 17.4±6.3 g. These two diets showed no difference, so the average was 16.55 g. However, the *Eup* and *Mor* diets were significantly lower than the two previous diets ($P < 0.05$), but identical to each other ($P > 0.05$), for 5.3±5.2 g and 7.0±4.0 g, respectively.

Table 3. Dry matter (DM), metabolizable energy (M.E), and crude protein (CP) daily voluntary ingestion, and rabbit does weight average daily weight gain (ADWG) during gestation and lactation periods

Physiological periods	Parameters	Diets					
		Pan			**Des**		
	VDIME (g/d DM)	122.0(16.1)[a]			122.4(8.6)[a]		
		P. max.	Pel.		*P. max.*	*D. tor.*	Pel.
Gestation		34.0(22.1)[de]	88.0		46.5(7.8)[d]	20.7(3.0)[b]	55.2
	VDIME (kcal/d)	391.50(18.5)[a]			390.44(18.5)[a]		
	VDICP (g)	17.5(2.3)[a]			17.6(1.2)[a]		
	Rabbit does ADWG (g/d)	15.7(6.1)[a]			17.4(6.3)[a]		
		Pan			**Des**		
	VDIME (g/d DM)	122.0±9.1[a]			131.2(8.3)[a]		
		P. max.	Pel.		*P. max.*	*D. tor.*	Pel.
Lactation		37.53(8.0)[f]	94.9		51.7(7.7)[d]	24.3(2.2)[b]	55.2
	VDIME (kcal/d)	425.98(17.7)[a]			418.28(17.7)[a]		
	VDICP (g)	17.7(1.3)[a]			18.8(1.2)[a]		
	Rabbit does ADWG (g/d)	-4.1(3.4)[b]			-11.9(3.0)[a]		
Physiological periods	**Parameters**	**Diets**					
		Eup			**Mor**		
	VDIME (g/d DM)	106.7(11.9)[b]			102.8(9.2)[b]		
		P. max.	*E. het.*	Pel.	*P. max.*	*M. ole.*	Pel.
Gestation		18.2(6.3)[e]	61.3(8.0)[a]	27.2	32.7(7.9)[de]	12.3(4.3)[c]	57.8
	VDIME (kcal/d)	341.74(18.5)[b]			327.96(18.5)[b]		
	VDICP (g)	16.3(1.8)[a]			15.4(1.4)[a]		
	Rabbit does ADWG (g/d)	5.3(5.2)[b]			7.0(4.0)[b]		
		Eup			**Mor**		
	VDIME (g/d DM)	117.1(7.6)[a]			102.8(9.2)[b]		
		P. max.	*E. het.*	Pel.	*P. max.*	*M. ole.*	Pel.
Lactation		19.9(5.0)[g]	70.1(5.5)[a]	27.2	47.3(6.3)[e]	17.7(3.0)[c]	57.8
	VDIME (kcal/d)	375.71(17.7)[b]			391.40(17.7)[b]		
	VDICP (g)	17.9(1.2)[a]			18.1(1.6)[a]		
	Rabbit does ADWG (g/d)	-9.8(2.2)[a]			-10.2(3.9)[a]		

Note. Results are given as Mean (SD), SD: standard deviation. The means in the same row followed by the same lowercase letter are not significantly different.

ADWG: Average daily weight gain; VDIME: voluntary daily intake of metabolizable energy; VDICP: voluntary daily intake of crude protein.

Ingredients: g/d: gram per day; Pel.: mixed type pellet for rabbits; *Pan. max.*: *Panicum maximum*; *D. tor.*: *Desmodium tortuosum*; *E. het.*: *Euphorbia heterophylla*; *M. ole.*: *Moringa oleifera*.

Diets: *Pan*: *P. maximum* + FACI© mixed type pellet for rabbits; *Eup*: *P. maximum* + *E. heterophylla* + FACI© mixed type pellet for rabbits; *Des*: *P. maximum* + *D. tortuosum* + FACI© mixed type pellet for rabbits; *Mor*: *P. maximum* + *M. oleifera* + FACI© mixed type pellet for rabbits.

[a], [b] Means within rows of diet with no common superscript differ ($P < 0.05$) by the Student Newman Keuls test.

3.2 Apparent Digestive Utilization Coefficient

Table 4 shows the apparent digestive utilization coefficients according to the diets. Its values were 56.6±5.3% for *Pan*; 62.6±5.3% for *Des*, 65.8±10.3% for *Eup*, and 62.8±8.9% for *Mor*. Compared to *Pan* diet, the green forages addition to *Pan* reference diet improved the coefficient. These apparent digestive utilization coefficients were significantly lower ($P < 0.05$) for *Pan* diet than those of *Des*, *Eup* and *Mor* diets. Within the green forage diets, the apparent digestive utilization coefficients did not differ significantly ($P > 0.05$), leading to a 63.73% average. So, adding the green forage to *Panicum* and rabbit mixed pellet ingredients increases the feed conversion ratios.

Table 4. Diets apparent digestive utilization ratio (%DM)

Designation	Diets			
	Pan	Des	Eup	Mor
Dry matter intake (g/kg DM)	115.6(5.9)[b]	126.8(8.2)[a]	117.6(6.2)[b]	116.0(11.6)[b]
Droppings dry matter (g/kg DM)	51.3(6.3)[a]	47.4(7.4)[a]	40.4(12.9)[a]	42.6(8.5)[a]
Apparent digestive utilization coefficient (%DM)	55.6(5.3)[b]	62.6(5.3)[a]	65.8(10.3)[a]	62.8(8.9)[a]

Note. Results are given as Mean (SD), SD: standard deviation.

[a], [b] Means within a row with different superscript differ ($P < 0.05$) by the Student Newman Keuls test.

In family rabbit farming, breeders are looking for a substantial feed reduction costs, by keeping the optimal animals' growth. If possible, this goal can be achieved by distributing coarse fodder, or whole green plants, or dried plants' parts (Kpodékon et al., 2009). So, Kadi et al. (2012) argued that it is important to know about plants' palatability for rabbits, their intake, and digestion levels. These apparent digestive utilization coefficients were close to Goby et al. (2017) findings. During their experiment, Goby et al. (2017) used fresh whole carrot, dry whole carrot, and alfalfa hay. They concluded that fresh whole carrot was better digested (85%) than dehydrated forage (56%). In addition, the dry matter ingested quantities from the alfalfa hay was 22% less than the carrot group dry matter intake. Thus, with this alfalfa hay, the intake was 25% below the rabbit's maximum ingestion capacity, which was between 70 and 80 g DM per day.

Nevertheless, although the rabbits had high digestion between 62.6 to 65.8% for *Des*, *Eup*, and *Mor*; and adequate intake 116 to 126.8 g DM per day for the same diets, the rabbit does weight loss (Table 3) were significant and higher than 9 g per day. This situation could be explained in part by an insufficient sulfur amino acid intake on average in experimental forages because green forage content for this nutrient is around 2% for 3.7% desirable intake. Also, weight losses were due to the high demand for both energy and protein in lactation (Bonnet, 2006). In Pan diet, the mineral deficits were corrected by the rabbit concentrated and mixed pellets. So, Pan diet rabbit does weights losses were minimized. These results agree with Kouakou et al. (2016) findings.

3.3 Rabbit Does Reproduction Parameters

After farrowing, rabbit does weights were 2966.9±174.2; 3032.4±167.3; 2957.8±159.1 and 2999.3±166.9 g, respectively for rabbit does fed on *Pan*, *Des*, *Eup*, and *Mor* diets (Table 5). Because we constituted homogeneous groups in weight before matting, these weights after farrowing did not differ significantly by diet ($P > 0.05$). Fertility rates were 66.6%; 83.3; 83.3% and 50%, respectively, for *Pan*, *Des*, *Eup*, and *Mor* diets. The number of pups born alive per diet was 96, 112, 112, and 76 from rabbit-does fed on *Pan*, *Des*, *Eup*, and *Mor* diets, respectively. According to Houindo (2002), rabbit does fertility is 61% in primiparous, and 50% in multiparous. Moreover, herein results were significantly higher than those of Houindo (2002), except for *Mor* diet (50%). As Toleba et al. (2017) findings, when they incorporated Neem leaves (*Azadirachta Indica*) in rabbit does' diets at an 8% incorporation rate, they got 100% females farrowing. *Pan* diet fertility result was lower than those of *Des* and *Eup* diets. Both diets improved *Pan* diet fertility by 25.07%, from 66.6% to 83.3%. Unfortunately, *Mor* diet depressed *Pan* diet fertility rate by 24.93%, from 66.6% to 50%. Altogether, *Des* and *Eup* diets outputs were 66.6% better than that of *Mor* diet.

Average litter sizes and average pups' weights were 6.0±1.8; 5.6±2.2; 5.6±1.1 and 6.3±2.1 rabbits, 50.2±8.9; 56.1±11.3; 54.3±12.7 and 49.2±11.8 g, respectively for *Pan*, *Des*, *Eup*, and *Mor* diets. These litter sizes and their weights did not differ significantly ($P > 0.05$). So, rabbit-does farrowed 5.87 pups, and the litter weighed 52.45 g in average. Similarly, Akpo et al. (2008) announced 5.7 pups per litter.

3.4 Milk Production

The milk production during the lactation peak period showed some variations. Indeed, peak lactation milk production under *Eup* diet (140.0±5.4) had a higher average milk yield than *Des* diet ($P < 0.05$). However, *Mor* diet production was median between *Eup* and *Des* (Table 6) and therefore did not differ significantly ($P > 0.05$) from either *Eup* or *Des*. In addition, rabbits on Pan diet produced significantly less milk than the other three diets ($P < 0.05$). Pups weight changes at peak lactation in *Des* and *Eup* diets were greater than those of *Pan* and *Mor* diets ($P > 0.05$). Thus, these two groups *Des* and *Eup* on one hand, and *Pan* and *Mor* on the other hand were similar in pairs ($P > 0.05$) to each other for this growth performance. Pups average weaning weights did not differ significantly. The pups daily weight gains at lactation peak were 6.3±0.3 g/d, 6.6±0.3 g/d, and 5.8±0.8 g/d for *Pan*, *Eup*, and *Mor* pups, respectively, and did not differ significantly ($P > 0.05$).

Table 5. Rabbit does reproduction parameters

Parameters	Diets			
	Pan	*Des*	*Eup*	*Mor*
Rabbit does number	24	24	24	24
Rabbit does weight at matting	3006.3(132.5)[a]	2999.0(140.9)[a]	3030.0(124.2)[a]	2990.7(126.4)[a]
Number of rabbit-does having farrowed	16	20	20	12
Rabbit does weight after farrowing (g)	2966.9(174.2)[a]	3032.4(167.3)[a]	2957.8(159.1)[a]	2999.3(166.9)[a]
Total pup number	96	112	112	76
Litter size	6.0(1.8)[a]	5.6(2.2)[a]	5.6(1.1)[a]	6.3(2.1)[a]
Pups' weights (g)	50.2(8.9)[a]	56.1(11.3)[a]	54.3(12.7)[a]	49.2(11.8)[a]
Fertility rate (%)	66.6	83.3	83.3	50

Note. Results are given as Mean (SD), SD: standard deviation.

[a], [b] Means within a row with different superscript differ ($P < 0.05$) by the Student Newman Keuls test.

Singularly, pups' weights from rabbit does fed on *Des* had the heaviest weights at peak lactation, and this weight was significantly higher than those of *Pan*, *Eup*, and *Mor* diets. At weaning period, *Pan*, *Des*, and *Eup* delivered the best average daily weight gain for 6.2±0.5, 6.4±0.6, and 6.4±0.4, respectively, compared to 5.4±06 g for *Mor* diet ($P < 0.05$). According to Kunnath et al. (2018), genetic group, birth season, and litter size have a significant influence on rabbit pups daily weight gain. This assertion is supported by Sherif (2018) findings. When Sherif (2018) used New Zealand white rabbits in Egypt, he got 27.2 g daily, which is 4.5 times higher than Cote d'Ivoire local breed performance. Similarly, when Omer et al. (2012) used some green forage, he got 30.36 g/d with New Zealand white rabbits. In relatively cool temperatures, the rabbits grow faster than under humid tropical conditions. So, this daily weight gain was distributed in a large interval. Moreover, feed presentation such as pellet, forage, or mash, and feed dietary lignin level affect significantly rabbit growth Gidenne et al. (2015). For example, when Akande (2015) added some roasted pigeon pea meal in the rabbit diet at 10, 20, and 30%, the daily weight gain decreased from 16 to 13, and 12 g, respectively. Again, Di-Meo et al. (2004) observed a daily weight gain between 13 and 14 g. Due to the high-temperature effect, Kunnath et al. (2018) got an average daily weight gain ranged from 8.86 to 29.52 g. So, the present experiment results were quite acceptable.

Table 6. Rabbit does milk production and pup rabbits' weight at peak lactation

Designations	Diets			
	Pan	*Des*	*Eup*	*Mor*
Daily rabbit does milk production (g)	109.6(4.9)[c]	126.6(5.1)[b]	140.0(5.4)[a]	131.5(6.6)[ab]
Pup weights at lactation pic (g)	187.4(2.1)[b]	195.7(3.2)[a]	192.0(2.0)[a]	185.6(1.6)[b]
Pup weaning weights (g)	230.7(5.4)	227.7(4.4)	236.7(2.5)	223.3(2.1)
Daily weight gain at lactation pic (g/d)	6.3(0.3)[b]	6.9(0.2)[a]	6.6(0.3)[b]	5.8(0.8)[b]
Pup daily weight gain at weaning (g/d)	6.2(0.5)[a]	6.4(0.6)[a]	6.4(0.4)[a]	5.4(0.6)[b]

Note. Results are given as Mean (SD), SD: standard deviation.

g/d: gram per day.

[a], [b] Means within a row with different superscript differ ($P < 0.05$) by the Student Newman Keuls test.

The feed composition is one of the main factors that influence rabbit does milk production. In addition to milk production quantity, its fat contents are also impacted (Pascual et al., 2003). Indeed, when Kowalska and Bielanski (2004) used the linseeds, the 4% linseed oil mixture did not only improve the milk production quantity, but it affected also the saturated fatty acid profile, which reflects a high fatty acids transfer rate. This close link between the feed fatty acid composition (especially omega 3) and milk has also been demonstrated by Castellini et al. (2004). Therefore, the best rabbit does milk production fed on *Des*, *Eup*, and *Mor* diets was probably due to these diets galactogenic activities (Oguike & Udeh, 2008). These plants galactogenic activities would be due to their ability to stimulate the secretion of the hormones, which could induce milk synthesis, particularly prolactin (Akouedegni et al., 2013). Indeed, polymers from plants including galacturonic acid and β-glucan are considered

hormones or hormone messengers in plants. This suggests that animal cells are also sensitive to these hormonal messengers (Sawadogo et al., 1989).

In addition, some plants used by farmers to increase milk production in cattle in Benin are mainly from leguminosae family, Moraceae, and Euphorbiaceae. Then, these plants use belonging to these families, are known in the traditional environment for their lactogenic power (Akouedegni et al., 2012). Undoubtedly, the daily milk quantities obtained from rabbits does fed on *Des*, *Eup*, and *Mor* diets for 126.6±29.1, 140.0±31.4, and 131.5±29.6 g, respectively, could be associated with these plants' intrinsic activities. In fact, they induce a good milk production, via the consequently prolactin secretion. According to Mosango (2008), and Sulistiawati et al. (2017), Euphorbia heterophylla and Moringa oleifera leaves' capsules contain phytosterol chemicals (poliferol and sterols), saponins, phenols, and terpenes including phorbolic diterpenes, whose play an important role in increasing prolactin levels. Indeed, prolactin plays an essential role in mammary gland growth and milk secretion induction (Akouedegni et al., 2013). An increase in this hormone concentration and an increase in lactocytes exposure duration to this same hormone will lead to an increase in the number of the receptors on the lactocytes membrane. Under these conditions, milk will be continuously secreted under the autocrine control of these lactocytes. Moreover, the milk production by these lactocytes will depend on the milk quantity they contain (Akouedegni et al., 2013). Similarly, these observations were made by Kiranawati and Nurjanah (2014), when they substituted Moringa oleifera leaf flour in experimental diets. Thus, in vivo in Wistar rabbits (*Rattus norvegicus*), they observed significantly more developed mammary glands later on, in contrary to the control congeners.

Desmodium tortuosum is still a little-known forage plant. Meanwhile, considering the dairy production performance of the rabbit does fed on it, *Desmodium tortuosum* is undeniably an excellent green forage. As Morris et al. (2014), during flavonoid concentrations determination, D. tortuosum produced 718 µg/g isorhamnetin. Screening *D. discolor*, *D. incanum*, *D. intortum*, *D. sandwicense*, and *D. tortuosum*, and their oil content and its fatty acid composition, Morris et al. (2014) concluded that *Desmodium* species could be used as alternatives livestock health products. According to Chibah-Ait et al. (2015), rabbit does milk production is important when the pup group size is large. Due to the temperature adverse effect on rabbit does milk production (Hue-Beauvais et al., 2015; Szendrö et al., 1999; Zerrouki et al., 2014), the present milk production results could have been better. In fact, Szendrö et al. (1999), Zerrouki et al. (2014), and Hue-Beauvais et al. (2015) demonstrated that rabbit does have difficulties in adapting to temperatures equal or higher than 30 °C, which significantly reduces their ability to produce milk. In addition, the lactogenic power attributed to certain plants would be due to nutritional intake and prolactin stimulating substances joint action in the pituitary gland (Adepo et al., 2010; Sepehri et al., 2000). According to Deleke Koko et al. (2011), specific lactogenic compounds plants are terpenes, steroids, flavonoids, and cardiotonic derivatives. Ouedraogo et al. (2004) stated that the conjugated chemical compounds groups action would be at the origin of the plants' galactogenic properties.

4. Conclusion

This work results show that *Desmodium tortuosum*, *Euphorbia heterophylla*, and *Moringa oleifera* associated with commercial rabbit pellet diets digestion is higher than 62%. While, the pellets should not be associated only with *Panicum maximum*, because this diet digestibility was 55%. Most importantly, these fresh weeds and commercial pelleted feed mixtures did not adversely affect the rabbits' performances. Specifically, commercial pellets for rabbits and *Panicum maximum* diets containing *Desmodium tortuosum*, *Euphorbia heterophylla*, and *Moringa oleifera* green forages had a very good galactogenic effect. So, *Desmodium tortuosum* and *Euphorbia heterophylla* were better than *Moringa oleifera* and could be used as green forages to improve milk production in local rabbit does breeding. It would be important to determine the active substances responsible for this galactogenic effect in these plants and to establish a correlation with milk production. Nevertheless, these results could be supported by assays on certain lactation hormones including prolactin and growth hormone in blood plasma. Also, blood cell counts, triglyceride, total and HDL cholesterols determination could provide some information on the animals' health status.

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Perceptions of Members of Households Regarding the Production and Marketing of Moringa (*Moringa oleifera*) in Thulamela Local Municipality

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Abstract

This study was carried out to determine how members of households perceive economic benefits of production and marketing of *Moringa oleifera* in the Thulamela Local Municipality. The study adopted the snowball sampling procedure to identify the population of members of households who produce and market Moringa. Simple random sampling procedure was adopted to select 146 participants. The Probit regression model was used as the analytical tool for this study. The results of the study revealed amongst others, that majority of members of households who were producing Moringa had no access to Moringa markets, and most of them were aware of the economic benefits of Moringa. The statistical significant variables which influenced the perceptions of members of households regarding economic benefits of production and marketing of Moringa were level of education ($p < 0.01$), Moringa farming experience ($p < 0.05$), access to market ($p < 0.05$), as well as the access to information ($p < 0.05$) about the production and the demand of Moringa produce. The study concluded that majority of respondents perceived that the production and marketing of Moringa would help to achieve sustainable livelihood for people living in Thulamela Local Municipality, while others were of the view that Moringa has the potential to improve nutrition, boost food security and foster rural development. The study recommended that establishment of Moringa markets, formation of Moringa cooperatives and promotional campaigns to educate members of households about the economic benefits of Moringa should be enhanced.

Keywords: economic benefits, marketing, moringa oleifera, production, thulamela municipality

1. Introduction

Moringa has gained much prominence due to its multiple uses and benefits for both agricultural and industrial development (Ashfaq et al., 2012). This attention often triggers investments and promotion campaigns to domesticate Moringa and establish large-scale commercial plantations (Achten et al., 2014). Moringa is a plant that is commonly used as a nutritional food supplement by some communities in South Africa. It has also been well approved as source of food (Pikade et al., 2013). Regarding one of the important reasons for studying development economics, is to understand how members of households can make transition out of poverty, understanding the perception of members of households regarding the improvement of Moringa production and market access are important approaches to rural development. This is because these two activities give members of households the opportunity to specialize and optimize their portfolios with respect to the available resources (Kamara, 2004).

The production and marketing of Moringa is increasingly recognised as an effective strategy for alleviating poverty and hunger, and it can be stated that a successful process of determining how members of households perceive the production and marketing of Moringa can help to promote inclusive and sustained economic growth and create decent work opportunities for members of households. According to Nadeau and Zakaria (2012) studies have equally shown that Moringa can provide excellent opportunities for agricultural producers, traders and processors, thereby making it effective in tackling micronutrient insecurity while equally holding the promise of sustainable economic returns to members of households. Moreover, Moringa production should

stimulate market participation by members of households who produce Moringa. The current emerging trends of economic potential of Moringa as a commercial tree species suggest why it could play a role as a leading edge in the 21st century as a veritable marketable product (Ajayi et al., 2013).

There has been an upsurge of interest in the cultivation and consumption of Moringa in South Africa and many health claims have been reported, and the government has encouraged members of households to cultivate Moringa trees in a drive to decrease malnutrition in many rural areas of South Africa (Hlophe, 2015). The burning desire to cultivate and consume Moringa has alarmed the government to embark on a campaign to encourage members of households to improve their production to commercial farming that brings about high Moringa yields and assist these households with the necessary production inputs. Taking into consideration that members of households expect immediate benefits from farming, any pathway towards sustainable Moringa production will necessarily require the inclusions of improved and predictable household productivity (Vanlauwe et al., 2014). This can only be possible by understanding how members of households view the expansion of Moringa production to a larger scale. Moreover, an analysis of sociocultural perceptions on Moringa would also provide further understanding of the behaviour of members of households regarding the tree (Gandji et al., 2018).

According to the case study compiled by NAMC (2011) commercial production of Moringa in South Africa is still at a very early stage, which makes it difficult to quantify the hectares under production, volume and value of the commodity. However, there are a few households producing the commodity as a food supplement. Empirical evidence from the study conducted by Omotesho et al. (2013) on the economics of its production highlights its potential as a tool for enhancing the income of its producers, as Moringa can be consumed and sold as a food source. This is expressing the importance of evaluating households' awareness and perception, as well as to explore more effective ways to increase its production and marketing, as most of members of households produce Moringa in their back yards, due to lack of income, knowledge and other necessary production inputs, such as improved seeds and fertilizers. According to Fadoyin et al. (2014) the productivity of Moringa has not grown sufficiently due to under-investment in new technology, slow adoption of existing improved technologies, constraints associated with investment climates and shortage of infrastructure.

Several studies have been conducted in exploring the use of Moringa for various industries (IDC, 2019). However, to date, only limited numbers of publications which seek to understand how members of households who produce Moringa can benefit from increasing Moringa productivity and marketing in rural areas are available. Therefore, this study will help to understand the major constraints which influences the perceptions of members of households regarding the production and marketing of Moringa, as well as examine how members of household perceived Moringa's production and marketing as a strategy for attaining socio-economic development in Thulamela Local Municipality.

2. Method

The study was conducted in the Thulamela Local Municipality, Vhembe District, Limpopo, South Africa. This is because the area is dominated by the emerging household Moringa farmers who do not have the resources to commercialize Moringa. The targeted population for this study was members of households who produce Moringa in the Thulamela Local Municipality. Simple random sampling procedure was adopted to select 146 participants for the study. Primary data was collected from members of households who produce Moringa using a designed research questionnaire. Close ended questionnaires and open-ended questionnaires were used to collect qualitative and quantitative data with the aid of thoroughly trained enumerator. The collected data was processed and examined to detect errors and omissions and corrected where possible. Based on the objectives of the study, the collected data was analysed using the IBM SPSS Statistics. Probit regression model was used as the main analytical tools for this study. Different analyses were computed to respond to the specific objectives of the study.

Specific objective, number one was to determine the socio-economic characteristics of members of households who produce Moringa in Thulamela Local Municipality. For this purpose, Cross-tabulation was used to examine the relationship between the socio-economic characteristics of members of households who produce Moringa and their level of awareness regarding the economic benefits of producing and marketing Moringa. The Test of Equality of Group Means was then used to compare two groups of members of households who produce Moringa and their respective means. In this case, members of households who are aware and those who are not aware of the economic benefits of producing and marketing Moringa were compared to find the dimensions that they differed on and each group was assessed for significance.

Specific objective number two was to determine the perceptions of members of households regarding economic benefits of production and marketing of Moringa in Thulamela Local Municipality. For this purpose, 4-point Likert scale was used to measure the perceptions of members of households regarding economic benefits of production and marketing of Moringa. Likert scale provides independence to a participant to choose any response in a balanced and symmetric way in either directions (Joshi et al., 2015). In this study, several statements were administered to members of households to allow them to choose their responses based on whether they strongly disagree, disagree, agree or strongly agree with the statement, and respondents were not allowed to select neutral options.

Specific objective, number three was to determine factors that influence perceptions of members of households regarding economic benefits of production and marketing of Moringa in Thulamela Local Municipality. The Probit model was employed to estimate the probability of members of households' perceptions regarding economic benefits of production and marketing of Moringa. The Probit model is a statistical probability model with two categories in the dependent variable (Liao, 1994; Uzunoz & Akcay, 2012). This study was interested on whether members of households were aware of the economic benefits of producing and marketing Moringa or not. The Probit model was more useful in this study because the dependent variable that was thought to be influenced was dichotomous.

The Probit model is a type of regression where the dependent variable can only take two values; the two possible outcomes in this study were aware and not aware denoted by 0 and 1. With reference to the study conducted by Krystalogianni et al. (2004), the Probit model in this study provides statistically significant findings of which the predictor variables increase or decrease the probability of members of households' awareness which is estimated as:

$$\Pr(P = 1|x) = \Pr(P = 1|x_1, x_2, \ldots x_k) \quad (1)$$

A variable P is defined so that:

$P = 1$ Aware;

$P = 0$ Not aware.

where, x denotes the full set of explanatory variables $(x_1, x_2, \ldots x_k)$ which is a vector of leading indicator series in the present study. Looking at first equation, the Probit model can be interpreted as:

$$\Pr(P = 1|x) = \mathrm{F}\left(\beta_0 + \beta_{1\times 1} + \ldots + \beta_{k\times k}\right) = \mathrm{F}(\beta_0 + x\beta) \quad (2)$$

where, F is a function taking on values strictly between zero and one, which ensures that the estimated response probabilities are strictly between zero and one; β is the set of coefficients corresponding to the indicator variables x. To make the Probit approach operational the probability of obtaining $P = 1$ is linked to an unobservable index I. The higher the value of the index I the more likely the members of households would be aware of the economic benefits of producing and marketing Moringa. The unobservable index I, which is required to be normally distributed for a Probit to apply, is determined by the set of explanatory variables x:

$$I = \beta_0 + x\beta \quad (3)$$

A threshold value is also required to indicate the possible occurrence of awareness. If the estimated I is greater than a threshold value I^*, then $T = 1$:

$$\Pr(T = 1|x) = \Pr\left(I^* \le I\right) = \Pr(I^* \le \beta_0 + x\beta) \quad (4)$$

The Probit model will estimate the coefficients β_0 and β and the unobservable series I. Once an estimate for I is obtained one can accept $P = 1$: (Awareness) if I is greater than I^*, the threshold value of I. The normality assumption for the unobservable index I means that one can obtain the probability that $I^* \le I$ from the standardized normal cumulative density function. Therefore:

$$\Pr\left(I^* \le I\right) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{I} e^{-t^2/2} \mathrm{d}t = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\beta_o + \beta x} e^{-t^2/2} \mathrm{d}t \quad (5)$$

Where, $p \sim \mathrm{N}(0,1)$.

The probability of a being aware $\Pr(\mathrm{P} = 1)$ is therefore measured by the area of the standard normal cumulative curve from $-\infty$ to I. Awareness, therefore, will be more likely to occur the larger the value of I. Equation 5 shows the probability that a standard normal variable (I in this case) will be less than or equal to the threshold I^*.

3. Results

This section presents the results of the socio-economic characteristics of members of households who produce Moringa, and their perceptions regarding the production and marketing of Moringa in Thulamela Local

Municipality.The findings of the study revealed that majority of members of households who were producing Moringa had formal education, and most of them were aware of the economic benefits of Moringa. The study found that there was less participation of youth in the production of Moringa in the study area, and majority of respondents were either married or in a cohabiting relationship.

The production of Moringa in the Thulamela Local Municipality was dominated by members of households who produced Moringa below the average of 53 kg per year, moreover, majority of them had less than 4 Moringa trees in their households. The study revealed that most respondents in the study area had knowledge and experience in the production of Moringa and they have been cultivating Moringa for more than 2 two years. Access to Moringa market was a major concern in the study area, members of households had negative perception about the production of Moringa due to lack of market. Most of the members of households in Thulamela Local Municipality received information about the production and the demand of Moringa from their friends and relatives due to poor access to extension services on Moringa.

Table 1. Description of variables

Variable
Dependent variable:
Awareness (0 = Not aware; 1 = Aware)
Independent variable:
Level of education **(X_1)**
1 = no formal education; 2 = primary level; 3 = secondary level;4 = tertiary level
Age group **(X_2)**
1 = 18-35 (Youth); 2 = 36-45 (Young adult); 3 = 46-60 (Adult); 4 = Above 61
Number of Moringa trees **(X_3)**
1 = less than 4trees; 2 = 5-10 trees; 3 = 11-15 trees; 4 = 16-20 trees; 5 = more than 21 trees
Moringa farming experience **(X_4)**
1 = less than a year; 2 = 1-3 years; 3 = 4-5 years; 4 = 6 years and above
Access to markets **(X_5)**
0 = no; 1 = yes
Access to information **(X_6)**
0 = no; 1 = yes
Gender **(X_7)**
0 = Female; 1 = Male
Marital Status **(X_8)**
1 = Single; 2 = Married; 3 = Divorced; 4 = Widowed; 5 = Cohabit

Note. N = 146.

Table 1 provides the general description of variables collected from members of households who are producing Moring in Thulamela Local municipality. The dependent variable in this study was the awareness of the economic benefits of producing Moringa. Eight (8) independent variables such as level of education, age group, number of Moringa trees, moringa farming experience, access to markets, access to information, gender, and marital Status were computed to test whether they have a significant impact on the awareness of economic benefits of producing Moringa.

Table 2. Test of equality of group means

Variables	*Not aware*	*Aware*	*Pooled*	*Wilk's λ*	*F*	*df1*	*df2*	*P-value*
EDU **(X_1)**	2.34	2.39	2.38	0.999	0.109	1	144	0.741
AGE **(X_2)**	2.93	2.38	2.55	0.941	8.976	1	144	0.003
NMT **(X_3)**	1.59	2.67	2.34	0.893	17.230	1	144	0.000
MFE **(X_4)**	1.89	2.29	2.17	0.964	5.348	1	144	0.022
ACMK **(X_5)**	0.09	0.33	0.26	0936	9.890	1	144	0.002
INFAM **(X_6)**	0.23	0.75	0.59	0.767	43.799	1	144	0.000
GEN **(X_7)**	0.50	0.46	0.47	0.999	0.187	1	144	0.666
MRTS **(X_8)**	3.48	3.07	3.19	0.986	1.979	1	144	0.062

Table 2 presented above measured the equality of group of means of independent variables between the members of households who are aware of the economic benefits of Moringa and those who are not aware. It can be observed from the test of group of equality of means that, there was a significant difference between socio economic variables at 1 percent, 5 percent and 10 percent level. A significant difference was observed at 1 percent level between independent variables such as Age group (AGE), number of Moringa trees (NMT) produced by members of households, access to information (INFAM) as well as access to Moringa market (ACMK). Moringa farming experience (MFE) showed a significant difference at 5 percent level, while the marital status (MRTS) showed a significant difference at 10 percent level.

Based on the results of test of groups of equality of means presented on Table 2 above, the age group mean scores of members of households who were aware of the economic values of Moringa (2.38) was lower than those who were not aware (2.93). These results also indicated that there was a statistically significant difference between group means at 1 percent. The significant difference implied that members of households who were not aware of the economic benefits associated with the production of Moringa were elderly people; this might be because most elders are not familiar with modern technologies compared to modern generation who can familiarise themselves with different communication channels which can assist them to gain knowledge on how Moringa can be utilised.

The results presented on Table 2 indicated that there was a significant difference in the mean scores at 10 percent level. The marital status mean scores of members of households who were not aware of the economic benefits of Moringa (3.48) was higher than those who were aware (3.07). Access to information showed a statistically significant difference in the mean scores of members of households who were aware and those who were not aware at 10 percent. The mean scores of access to information showed that members of households who were aware of the economic values of Moringa had a greater mean (0.75) compared to their counterpart (0.23).

Table 3. Parameter estimates of the Probit model

Variables	Coefficients	Std. Error	*z*-Statistics	P-value
Level of education **(X_1)**	-1.053*	0.227	-4.643	0.000
Age group **(X_2)**	-0.644	0.414	-1.555	0.120
Number of Moringa trees **(X_3)**	-0.686	0.511	-1.342	0.180
Moringa farming experience **(X_4)**	1.198**	0.511	2.343	0.019
Access to markets **(X_5)**	2.180**	1.063	2.050	0.040
Access to information **(X_6)**	1.364**	0.693	1.968	0.049

Note. *p < 0.01; **p < 0.05; N = 146.

Table 3 above presents the estimated coefficients for Probit regression equations. The Probit model was employed to estimate the probability of members of households' perceptions regarding economic benefits of production and marketing of Moringa. In this case, the study was interested on whether or not the perceptions of members of households were influenced by predictor variables. The estimated coefficients and standard error showed which factors influence the perceptions of members of households regarding the production and marketing or Moringa. The Probit model in this study was significant at 1 percent level of probability, and the likelihood test statistic results of the model showed that access to market, Moringa farming experience and access to information were statistically significant at 1 percent and 5 percent level of probability. The results also showed that level of education has negative and statistically significant (1 percent) effect on awareness of economic benefits of producing and marketing Moringa.

Table 4. The rising demand of Moringa

The rising demand of Moringa can help to achieve sustainable livelihoods							
			Strongly disagree	Disagree	Agree	Strongly agree	Total
Awareness of economic benefits of Moringa	Not Aware	N	1	2	29	12	44
		Sub-total	0.7%	1.4%	19.9%	8.2%	30.1%
	Aware	N	0	2	51	49	102
		Sub-total	0.0%	1.4%	34.9%	33.6%	69.9%
Total		N	1	4	80	61	146
		Total	0.7%	2.8%	54.8%	41.8%	100.0%

Source: Survey data (2018).

Table 4 above indicated that most (34.9 percent) of members of households who were aware of the economic benefits of producing Moringa in the study area agreed that the rising demand of Moringa products can help to achieve sustainable livelihoods for people living in rural areas, followed by 33.6 percent of respondents who strongly agreed to the same statement. The study conducted by Mabapa et al. (2017) found that Moringa production has a high commercial value thus making its cultivation a potential cash earning opportunity that can enhance the livelihoods of rural dwellers in the province. The study revealed that about 36.3 percent and 33.6 percent of respondents who were aware of economic benefits of Moringa agreed and strongly agreed that Moringa has the potential to improve nutrition, boost food security and foster rural development. Only 1.4 percent of members of households disagreed with the statement.

Table 5. Potential of Moringa

Moringa has the potential to improve nutrition and boost food security							
			Strongly disagree	Disagree	Agree	Strongly agree	Total
Awareness of economic benefits of Moringa	Not aware	N	0	2	29	13	44
		Sub-total	0.0%	1.4%	19.9%	8.9%	30.1%
	Aware	N	0	0	53	49	102
		Sub-total	0.0%	0.0%	36.3%	33.6%	69.9%
Total		N	0	2	82	62	146
		Total	0.0%	1.4%	56.2%	42.5%	100.0%

Source: Survey data (2018).

The table presented above (Table 5) showed that there is a need to intensify campaigns which educate members of households about the importance and potential of Moringa in rural livelihoods, this is because within 30.1 percent of members of households who were not aware of the economic benefits of producing Moringa, 19.9 percent indicated that they agree to the statement that Moringa has the potential to improve nutrition, boost food security and foster rural development. This was also affirmed by the paper published by Agbogidi and Ilondu (2012) that expansion of Moringa production will significantly contribute to food security thereby, alleviatingpoverty and improving rural health care.

Table 6. Improving Moringa productivity

Improving Moringa productivity should be linked with accessible markets							
			Strongly disagree	Disagree	Agree	Strongly agree	Total
Awareness of economic benefits of Moringa	Not aware	N	1	1	25	17	44
		Sub-total	0.7%	0.7%	17.1%	11.6%	30.1%
	Aware	N	0	3	50	49	102
		Sub-total	0.0%	2.1%	34.2%	33.6%	69.9%
Total		N	1	4	75	66	146
		Total	0.7%	2.8%	51.4%	45.2%	100.0%

Source: Survey data (2018).

Improving productivity of Moringa should always be associated with accessible market for the accumulation of profit. This statement was supported by 34.2 percent and 33.6 percent of members of households who were aware of the economic benefits of producing Moringa in the Thulamela Local Municipality. In the study conducted by Omotesho et al. (2013) market uncertainty was found to be one of the major challenges to the large-scale cultivation of Moringa in developing countries. This implied that accessible Moringa market is crucial in the advancement of Moringa. The results presented above (Table 6) also indicated that among 30.1 percent of respondents who were not aware of economic benefits of Moringa, 17.1 percent 11.6 percent agreed and strongly agreed respectively to the statement that improving productivity of Moringa should always be associated with accessible market for moringa. A total of 51.4 percent and 45.2 agreed and strongly agreed with the same statement respectively, while only 0.7 percent and 2.8 percent of respondents disagreed and strongly disagreed with the statement.

4. Discussion

As noted in Table 3 above, members of households who had no formal education were less likely to be aware of the economic benefits of producing and marketing Moringa. These results implied that the level of education had a negative significant impact on the perceptions of members of households regarding the production and marketing of Moringa in the study area. It was affirmed by the study conducted by Azeez et al. (2013) which revealed that Moringa farmers that had a primary education were more technically efficient in Moringa production than those with no formal education.

Moringa farming experience was one of the factors influencing the perceptions of members of households regarding the production and marketing of Moringa in the study area. The results of the study presented on Table 3 showed that members of households who had knowledge and experience in the production and marketing of Moringa were more likely to be aware of the economic benefits of producing and marketing Moringa. These results revealed that the farming experience in Moringa production had a positive impact on how members of households perceived the production and marketing of Moringa in the Thulamela Local Municipality.

The results of the study revealed that perceptions of members of households on Moringa production and marketing was influenced by access to its market. As per the results of the study (Table 3), members of households who had access to Moringa markets where more likely to be aware of the economic benefits they can derive from the production and marketing of Moringa. The study conducted by Mabapa et al. (2017) found that most farmers are willing to expand their Moringa production, but they are finding it difficult due to lack of effective market. This implies that members of households had positive perceptions on how they can utilise Moringa for socio economic benefits, therefore, the challenge of effective market access has to be addressed.

The results of the study presented on Table 3 showed that members of households who had access to information about the demand of Moringa were more likely to be aware of the economic benefits of producing and marketing Moringa. These results implied that access to information about the demand of Moringa by members of households had a positive significant impact on how members of households in the Thulamela Local Municipality perceived the production and marketing of Moringa.

5. Conclusion

From the results of the study, it can be concluded that members of households had positive perception about the production and marketing of Moringa in Thulamela Local Municipality as majority of respondents perceived that the production and marketing of Moringa would help to achieve sustainable livelihood for people living in Thulamela Local Municipality, while others were of the view that Moringa has the potential to improve nutrition, boost food security and foster rural development. It can also be concluded that, members of households who had no formal education were less likely to be aware of the economic benefits of producing and marketing Moringa. Furthermore, members of households who had access to information about the demand of Moringa were more likely to be aware of the economic benefits of producing and marketing Moringa. The production of Moringa in the Thulamela Local Municipality was mainly dominated by members of households who produced Moringa below average.

6. Recommandations

To effectively increase the production and marketing of Moringa in Thulamela Local Municipality, the study recommended that establishment of Moringa market, formation of Moringa cooperatives and promotional campaigns to educate members of households about the economic benefits of Moringa should be enhanced. Members of households who are aware of the economic benefits of Moringa should be encouraged through the provision of necessary inputs. Further studies should be done on similar topic to address the gaps.

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Reduced Asian Soybean Rust Control by Commercial Fungicides Co-formulations in the 2018-2019 Growing Season in Southern Brazil

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Abstract

It has been a growers concen the reduction of Asian soybean rust (ASR) control by commercial fungicide co-formulations in the last growing seasons in southern Brazil. The objective of this work was to assess the ASR control efficacy by the most used co-formulations in the 2018/19 season. In a field experiment, 19 fungicides in commercial formulations to control soybean rust caused by *Phakopsora pachyrhizi*, were evaluated. Chemicals at their recommended doses were sprayed at four soybean growth stages. The first application was performed with 1.82% leaflet incidence and coinciding with R1 phenological stage. The others were performed at 14-18 days intervals. At stage R6, end of the epidemic and coinciding with half of the defoliation in the control plots, the leaf severity was appraised. The experiment was conducted with Ativa soybean cultivar, in 3 × 6 m plots, four replications and randomized block design. The harvest was made with a plot combine and the yield expressed in grains kg/ha. The means were compared by the Scott-Knott test. The disease control efficacy by 17 fungicide co-formulation showed control less than 57%, one with 78% and none with ≥ 80%. The unsprayed treatment severity was 81% and the greatest control of 78% resulted in 3,876 kg/ha yield. Therefore, the hypothesis raised in this work was accepted showing that the site-specific fungicides co-formulations are showing efficacy reduction season after season.

Keywords: fungicide resitance, *Glycine max*, *Phakopsora pachyrhizi*

1. Introduction

Soybean [*Glycine max* (L.) Merr.] growing area in Brazil, has increased season-after-season, reaching in 2018/19 35.8 milion hectares (CONAB, 2019).

Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi* (H. Sydow & P. Sydow, 1914), was first reported in March 2001/02 season in Paraguay and in May in Brazil (Yorinori et al., 2001) 18 years ago, beeing since than the major crop disease with the highest damage.

The decision making for disease control with fungicides is based on its amount (Danelli et al., 2015) and on the caused damage, sensu Nutter et al. (1993). The damage caused by ASR can be appraised on commertial farms through the function: y = 1,000 – 6.7 LI, where, y is grain yield normalized to 1,000 kg/ha and LI is leaflet incidence (Danelli et al., 2015).

Aiming at to reduce the damage, among the strategies to control ASR, the most efficient and praticed by growers is fungicides sprayings that, when efficient have potencial to reduce crop damage and loss (Nutter et al., 1993) and cover the application cost (Reis et al., 2019). Nevertheless, their use increase production cost and are showing reduced efficacy due to the fungus sensitivity reduction towards site-specific mode of action (MOA) fungicides (Silva et al., 2008). .

The repeated spraying of the same site-specific mecanism of action (MOA) to control a high risk fungus for resistance development, and with a high sporulation potential in a large growing area, with many sprayings per season (up to 10 are still performed), have accelerated the sensitivity reduction of *P. pachyrhizi* towards fungicides (Ishii & Hollomon, 2015; Reis et al., 2017).

In the 2003/2004 growing season, the cooperative experimental fungicides net was implemented with commertial fungicides in several sites of country. The ASR control results have shown a *P. pachyrhizi* sensitivity reduction against the three site-specific fungicides used in its control: DMIs, QoIs, and SDHIs. The early control

efficacy reduction (< 80%) has worried researchers to search for solutions to recover fungicdes eficacy from < 50% to > 80%.

The ASR control failure in Brazil was first reported six seasons after the begginning of rust control with the DMI tebuconazol (Fundação MT em Campo, 2008; Silva et al., 2008; Reis et al., 2017). In the first seasons of its use solo, control was > 80% and in the 2027/18 season, but in the national fungicide trials, its efficacy was reduced to 22% (Reis et al., 2017). Since than on, the evolution of the control reduction season-after-season tor the three MOA site-specific DMIs, QoIs, and SDHIs has been reported and did not stop yet. Co-formualations containing DMI, QoI and SDHI, in double or triple mixes, have been sprayed in a large soybean grown area without the additon of multi-sites, the main reason for the reduction of fungicides efficacy.

The hypothesis raised in this work was that the site-specific MOA fungicides co-formulations are showing efficacy reduction each season reaching in the last < 50% control.

Objective of this work was to assess the ASR control efficacy by commertial co-formulations in the 2018/19 season in Southern Brazil.

2. Materials and Methods

The experiment was carried out at Fazenda Carvalho ERS—324, Km 69, Passo Fundo/RS, (28°12′18″ latitude, 52°29′45″ longitude, and altitude 660 m a.s.l.), during 2018/19 growing season.

Brasmax Ativa RR, soybean cultivar was directed sown under black oats residues on December 10, 2018, with 50 cm row spacing, and 25 seeds/m^2.

Crop fertilization (300 kg/ha of 02-20-30; N-P_2O_5-K_2O), herbicides and insecticides applications were according to technical recommendations.

Fungicides (Table 1) sprayings were performed with a back-pack sprayer CO_2 driven pressure, boom containing six Teejet model XR 110015 nozzles and water volume 150 L/ha.

Table 1. Fungicides description and doses

Fungicide	Abbreviation	Mechanism of action	Concentration (a.i.)	Dose (mL or kg/ha)
Difenoconazole + cyproconazole	Dif + cypr	DMI + DMI	75 + 45	0.3
Azoxystrobin + cyproconazole	Cypr + azox	DMI + QoI	60 + 24	0.3
Fenpropimorph	Fenp	IBE	225	0.3
Trifloxystrobin + cyproconazole	Trif + cypr	QoI + DMI	75 + 32	0.2
Picoxystrobin + cyproconazole	Pico + cypr	QoI + DMI	60 + 24	0.3
Carbendazim + tebuconazol + kresoxim methyl	Carb + tebu + kres	TSI + DMI + QoI	25 + 125 + 156.25	1.25
Trifloxystrobin + tebuconazole	Trif + tebu	QoI + DMI	50 + 100	0.5
Trifloxystrobin + porthioconazole	Trifl + prot	QoI + DMI	60 + 70	0,.4
Metominostrobin + tebuconazole	Meto + tebu	QoI + DMI	79.75 + 119.63	0.725
Azoxystrobin + benzovindiflupyr	Azox + benz	QoI + SDHI	60 + 30	0.2
Picoxystrobin + tebuconazole	Pico + prot	QoI + DMI	60 + 100	0.5
Trifloxystrobin + prothioconazol + bixafen	Trif + prot + bix	QoI + DMI + SDHI	75 + 87.5 + 62.5	0.5
Pyraclostrobin _ epoxiconazole + fluxapiroxade	Pyra + epox + flux	QoI + DMI + SDHI	65 + 40 + 40	0.8
Pyraclostrobin + fluxapiroxade	Pyra + flux	QoI + SDHI	116,55 + 58.45	0.35
Tebuconazole + chlorothalonil	Tebu + chlo	DMI + multissite	125 + 1,125	2.5
Azoxystrobin + cyproconazole + mancozeb	Azox + mcz + cypr	QoI + multissite + DMI	90 + 60 + 1.350	2.0
Picoxystrobin + benzovindiflupyr	Pico + benz	QoI + SDHI	60 + 30	0.6
Picoxystrobin + tebuconazol + mancozeb	Pico + tebu + mcz	QoI + DMI + multissite	66.5 + 83.33 + 1.000	2.5

The experimental design was a randomized block with 19 treatments, four replications, with 3.5 m (6 rows) wide and 6.0 m long corresponding to 21.0 m^2 plots.

For rust assessment two pathometric methods were used: (a) For rust detection in the experimental area, weekly from V5 GS, five plants from the experiment borders were collected. Only central leaflets with leaves petioles inserted in the main plant stem were removed and assessed according to Ogle et al. (1979) and rust incidence was assessed under stereo microscope (30-50x); (b) The treatments effects on rust control were assessed based on leaflet severity. Severity notes, considering the percent leaflet area with symptoms/signs, were assigned in the central row according to Godoy et al. (2005).

At plant ripening, 12 m^2 area/plot was harvested with a Massey Ferguson adapted plot combine, grains were cleaned, moisture content adjusted to 13%, and grain yield appraised to kg/ha.

Collected data were submitted to test of normality, analyses of variance, regression and means compared Scott & Knott test.

3. Results and Discussion

Regarting the 2018/19 season, ASR was detected on January 16th, at V5 GS, 37 days after sowing on December 10th, 2018. In 43 days epidemics reached 100% leaflet incidence at R4 GS (Figure 1).

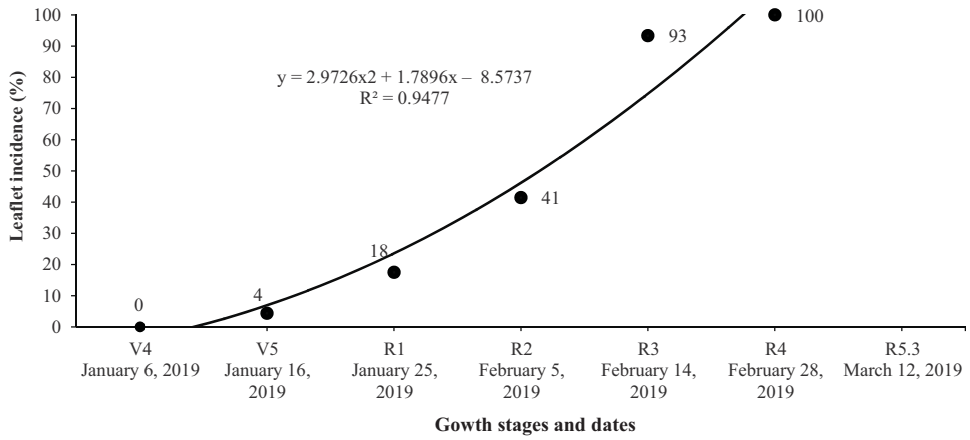

Figure 1. Progress curve of Asian soybean rust rated by leaflet incidence

The data related to the effect of the treatments on leaflet severity, means compared by the Scott & Knott test and with coefficient of variation of 5.91% are shown in (Figure 2). The epidemic intensity in unsprayed plots reached 81% considered severe but among the means registered in previous sesons. The 18%, the lowest severity, was quantified in the treament with picoxystrobin + tebuconazol + mancozeb a multissite fungicide. On the other hand, in treatment with just picoxystrobin + tebuconazol without mancozeb control was 49% which shows the increasing fungitoxicity by the multissite addition. This finding shows the benefical effect of the multissite mancozeb to fight ASR resistance (Figure 3). Among the tested fungicides only three have multi-site in their co-formulation: chlorothalonil + tebuconazole, azoxystrobin + cyproconazole + mancozeb, and picoxystrobin + tuboconazole + mancozeb.

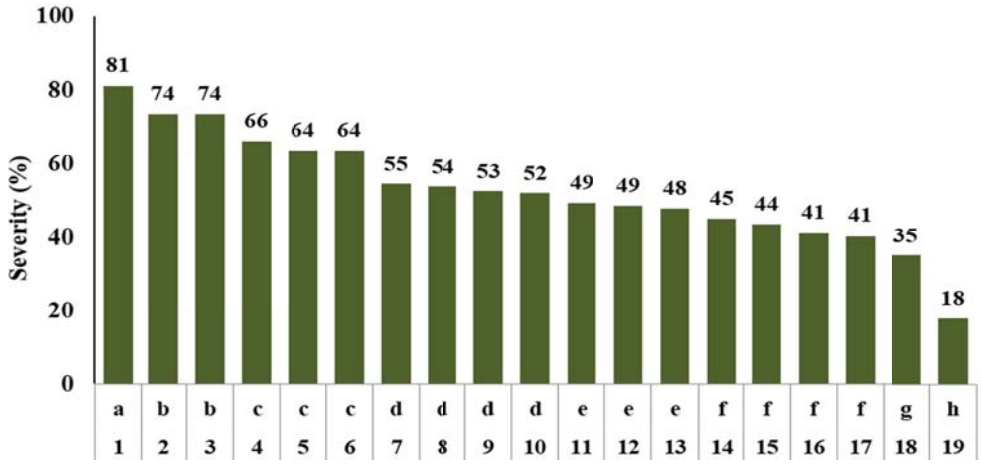

Figure 2. Effect of treatments on Asian soybean rust leaflet severity at R6 GS. Means (letters at columns base) compared by Scott & Knott test. Treatments: (1) Unsprayed; (2) dife + cypr; (3) azox + cypr; (4) fenp.; (5) trifl +cypr; (6) carb + tebu + kres; (7) trifl + tebu; (8) trif+ prot + bixa; (9) meto + tebu; (10) azox + cypr; (11) azox + benz; (12) pico + tebu; (13) trifl + prot; (14) pyra + epox + flux; (15) pyra + fluxa; (16) chlo + tebu: (17) azox + cypr + mcz; (18) pico + benz; (19) pico + tebu + mcz

Only three fungicides showed control higher than 50%, five between 40 to 49%, five between 33 to 39%, two with 22%, and two with 9%.

The evolution of the control reduction was analysed by Reis et al. (2017). In the 2005/05 seasons the control by 'tebu' was 90-91%, in the 2007/08 by 'cyp' + 'azox' was 86%, in the 2009/10 by 'epox' + 'pyra' was 79% and, in the 2009/10 by 'azox' was 79%. Therefore, comparing those control with the obtained in the present study, the reduced control in the last season was clearly shown (Figure 3).

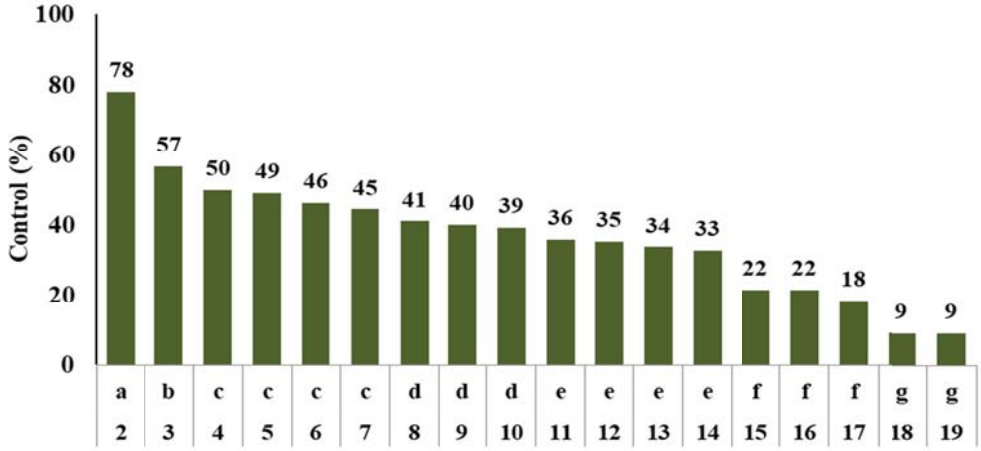

Figure 3. Treatments effect on ASR final leaflet severity control at R6 GS. Means (letters at columns base) compared by Scott & Knott test. (2) pico + tebu + mcz; (3) pico +benz; (4) azox + cypr + mcz; (5) chlo + tebu; (6) pyra + flux: (7) pyra + epox + flux; (8) trifl + prot: (9) pico + tebu; (10) azox + benz; (11) pico + cypr; (12) meto + tebu; (13) trifl + prot + bixa; (14) trifl + tebu; (15) trifl + cypr; (16) carb + tebu + kres; (17) femp; (18) dife + cypr; (19) azox + cypr

The quality and timing of fungicides spraying in field experiments are more precise than in commercial crops, therefore, the results obtained in the field may be inferior to those obtained here. The low control obtained in commercial farms, even where multisite is applied, presents low efficacy by not using the recommended dose (*i.e.*, mancozeb 1.5 kg/ha), not in all sprayings (national average of 2.6/ha). The maximum yield control is achieved with > 80% efficacy (Reis et al., 2019). Spraying site-specifics in double or triple co-cormulations, with cross and mutiple resistance, in all sprayings/area, and in most of the growing area is increasing the *P. pachyrhizi* sensitivity reduction to these fungicides threatening the economically sustainable control.

In the experiment, the relationship between grain yield (y) and ASR leaflet severity (x) was represented by the equation $y = -19.46x + 4{,}421.1$ and $R^2 = 0.8244$ (Figure 4). Therefore, for each severity percent point there was a grain yield reduction of 19.46 kg for 4,421.1 kg/ha potential yield. With this equation one can calculate the economic damage threshold according to Munford and Norton (1984) for any grain yield and rust severity, to time the first fungicide spraying as performed in the present work (Danelli et al., 2015).

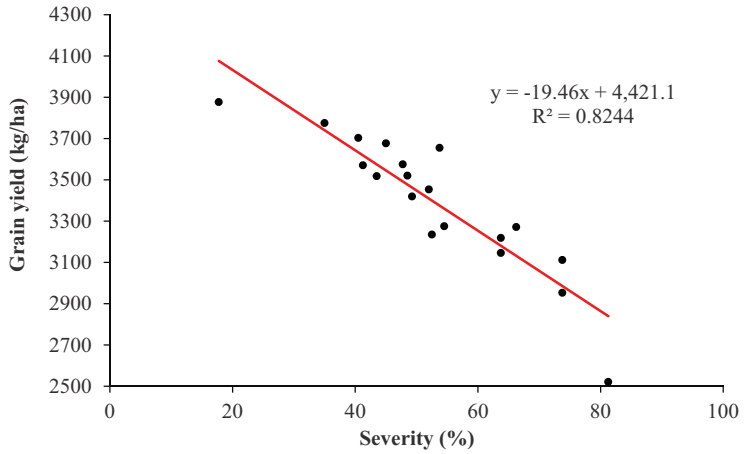

Figure 4. Negative relationship between soybean grain yield and Asian soybean rust leaflet severity

ASR damage, sensu Nutter et al. (1993), was calculated by the difference between the highest yield (3,876 kg/ha) and the yield in each treatment. The negative relationship between damage (kg/ha) and ASR control (%) was represented by the function y = -13.45x + 931.12 (R^2 = 0.819). For each percent point control reduction there was a 13.45 kg grain reduction for a maximum 931.12 kg/ha damage (Figure 5). The highest yield 3,826 kg/ha was achieved with 78% control and considered without damage (Table 2). Thus, taking for example the lowest and the highest fungicides control in Figure 3, damage can be appraised with the generated function: for 9% control, 810.07 kg damage, and for 57% control, 164.47 kg reduction.

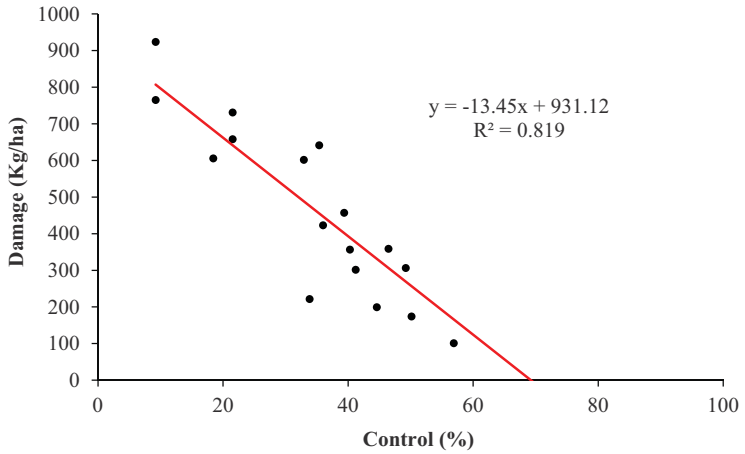

Figure 5. Negative relationship between Asian soybean rust damage and control

Related to the damage caused by plant diseases most of the published papers quantified the yield reduction only by adjectives instead of numbers and the used methodology for damage quantification is not described. The damage can be appraised knowing the relationship between different disease intensities and the resulting damage (Sah & MacKenzie, 1987). In our study, considering the maximum ASR severity of 83%, the maximum yield 3,876 kg/ha and the unsprayed treatment yield of 1,355 kg/ha, therefore the reduction was 1,355 kg/ha or 34.95%, lower than those mentioned in the literature. Each severity point reduced 17.0 kg in a 3,876 kg/ha actual gross yield.

In the first spraying cost was considered soybean kneading by sprayer wheels in commercial farms (32 m long boom, 35 cm width tires), fuel (75 HP engine), labor, fungicide price (dose/ha), yield potential kg/ha), soybean prince (one US$ = R$ 5.20; US$ 26.47/60 kg, Dec., 2020—Cotrijal, Não Me Toque county, Rio Grande do Sul state, Brazil), totaling US$ 46.00/ha, or 104 kg/ha soybean. In the calculation the costs were transformed into soybean grains weight (kg/ha) (Table 2).

Table 2. Asian soybean rust spraying cost, and treatments effect on control, damage, and grain yield

Treatments	Fungicide cost			Total cost[v] (Kg/ha)	Control (%)	Damage[x] (Kg/ha)	Yield (Kg/ha)	
	Dose (Kg/ha)	US $/ha	Soy (Kg/ha)				Gross[y]	Net[z]
Unsprayed	0	0	0	0	0	1355	**(2521)** f	1355
Dife + cypr	0.3	9.03	20.43	411	9 g	765	3111 d	2700
Fenp	0.3	8.37	18.91	397	18 f	606	3271 d	2874
Chlo + tebu	2.5	20.19	45.63	643	49 c	306	3570 c	2927
Pico + cypr	0.3	9.81	22.17	427	36 e	423	3453 c	3027
Azox + cypr	0.3	8.13	18.38	392	9 g	924	2953 e	2561
Trifl +cypr	0.2	9.51	21.51	421	22 f	731	3145 d	2725
Trif + tebu	0.5	7.79	17.60	385	33 e	602	3275 d	2890
Meto + tebu	0.725	11.85	26.77	469	35 e	642	3235 d	2766
Pico + tebu	0.5	15.38	34.77	543	40 d	357	3520 c	2977
Carb + tebu + kres	1.25	18.04	40.67	598	22 f	658	3218 d	2621
Trifl + prot	0.4	20.98	47.42	659	41 d	301	3575 c	2916
Azox + benz	0.2	16.23	36.68	560	39 d	457	3419 c	2859
Pico + benPico + benz	0.6	22.50	50.85	691	57 b	101	3775 a	3085
Pyr + flux	0.35	22.42	50.68	689	46 c	359	3517 c	2829
Pyra + epox + flux	0.8	23.13	52.28	704	45 c	200	3677 b	2973
Azox + cypr + mcz	2.0	20.54	46.42	650	50 c	174	3702 b	3053
Trif+ prot +bixa	0.5	28.85	65.19	823	34 e	222	3655 b	2832
Pico + tebu + mcz	2.5	29.71	67.15	763	**78 a**	0	3876 a	3036
C.V. (%)					10.6		2.8	

Note. [v]Total cost: kneading by sprayer wheels, fuel, labor, fungicide price (four spraying/ha), yield potential kg/ha, soybean price (US$ 26.47/60 kg); [x]Damage: the highest gross yield 3,876 kg/ha minus the yield in each treatment; [y]Gross yield: actual yield; [z]Net yield: Gross yield minus the spraying cost (kg/ha). Means follow by the same letter in the columns do not differ by Scott-Knott's test.

Our work shows that the profit is relate to the control efficacy and cost of application (Table 2).

In the last growing seasons experiments have shown a reduction in the efficacy of Asian soybean rust control by commercial fungicides (Reis et al., 2017). This statement can be confirmed by the mixtures performance with control less than 50% (Figure 3). Most of the commercial chemicals used by growers do not contain in their formulation multisite such as chlorothalonil, mancozeb or copper oxychloride (Figure 2), and there is a need for tank mixing which is little used. This has delayed the use of multisite across the soybean grown area and in all sprayings. Therefore, there is a need to shorten the time for all fungicides sold for soybean rust control to contain multisite in their formulation, as happened with those used to potato, tomato and grapevine downy mildew control (Duvauchelle & Ruccia, 2015).

The hypothesis was not accepted only for two fungicides that showed control higher than 50%.

It may be concluded that, season after season, the sensitivity of *P. pachyrhizi* to site-specific fungicides is decreasing. One should keep in mind that the maximum yield is obtained with control > 80%.

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Antagonism of Plant Pathogens by *Calotropis procera*

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Abstract

Phomopsis sojae and *Sclerotinia sclerotiorum* are responsible for stem and pod dryness and white mold in soybean. These pathologies directly affect the quality of seeds/grains and compromise the entire plant. The use of extracts from different plants has been the subject of research for the control of several phytopathogens. *Calotropis procera* is among botanical species that synthesize efficient compounds for biocontrol. In this context, the aim of this study was to evaluate the *in vitro* effect of *C. procera* aqueous extract on *P. sojae* and *S. sclerotiorum*. The experiment was carried out in completely randomized blocks in a 2 × 5 factorial scheme (two fungi and five extract concentrations 0%, 5%, 10%, 15% and 20%) with 4 replicates. *C. procera* aqueous extract concentrations were added to Petri dishes containing PDA. After 48 hours, the mycelial growth rate was evaluated. After seven days of incubation, the fungal colony area, sporulation, and germination of *P. sojae* and *S. sclerotiorum* were evaluated. There was significant interaction between fungi × extract concentrations ($p < 0.05$) for all variables analyzed. The mycelial growth rate of *P. sojae* was lower than that of *S. sclerotiorum*. The diameter of the *P. sojae* fungal colony was smaller than that of *S. sclerotiorum* when concentrations of 5%, 10% and 15% were used. As the extract concentration increased, fungi sporulation and germination reduced.

Keywords: stem and pod dryness, white mold, silk cotton, plant extracts

1. Introduction

Soybeans (*Glycine max* L.) belongs to the Fabaceae family and is an annual cycle plant with determined, indeterminate and semi-determined growth habit. It is one of the main oilseeds grown worldwide (Oliveira & Hecht, 2016). Soy is rich in protein and oil, 40% and 22%, respectively (Pratap et al., 2016), which makes it an important raw material for human and animal nutrition (Voora et al., 2020), currently being one of the most traded commodities, with numerous uses (SOYSTAT, 2016). In 2020, Brazil produced about 133 million tons, followed by the USA (116 million tons), Argentina (53 million tons) and China (17 million tons), being the world's largest producer (FAO, 2020).

Several plant pathogenic fungi (Hosseini et al., 2020; Haddad et al., 2017) affect soybean crop, among which *Phomopsis sojae* and *Sclerotinia sclerotiorum* stand out. These phytopathogens cause dry stem (Hosseini et al., 2020) and white mold (Pawlowski et al., 2019) respectively. *P. sojae* causes production losses by affecting seeds/grains (Mena et al., 2020). *S. sclerotiorum* attacks the entire plant by depositing its mycelium (Ranjan et al., 2018). It is known that diseases are one of the factors that limit the production of many plants worldwide (Kumar et al., 2016). The use of agrochemicals to control these two fungi in soybeans is an effective and successful approach (Willbur et al., 2019). However, the large-scale use of agrochemicals has resulted in problems such as environmental pollution, decreased biodiversity, and resistance to pathogens, among others (Haq et al., 2020).

Sustainable management systems are essential tools to maintain yield over the years. In these systems, plants can be protected from disease with environmentally friendly tools, low impact on production and environment (Acosta-Motos et al., 2020). Plants produce several metabolites that can act against phytopathogens (Mishra &

Arora, 2018). The use of plant extracts to control phytopathogens has been widely studied (Shuping & Eloff, 2017; Palou et al., 2016; Zaker et al., 2016). Plant extracts have been proven to be rich in bioactive compounds and antioxidants (Choudhury et al., 2018). The advantage of using natural extracts is that they can be prepared by the farmer himself as an alternative in the management of diseases (Isman, 2017).

The botanical species *Calotropis procera*, also known as silk cotton, is a plant with wide geographical distribution (Yoganandam et al., 2019). It contains bioactive compounds such as phenols, polysaccharide terpenes and flavonoids (Gawade et al., 2017). Due to its numerous antifungal properties (Khan et al., 2019), the species has been extensively studied (Mohamed et al., 2017; Cavalcante et al., 2016). Ali et al. (2020) found that *C. procera* inhibits the *in vitro* mycelial growth of *Alternaria alternata*. Unlike chemical botanical fungicides, offer better protection for crop, soil and environment. In view of the need for alternative control of these phytopathogens and the possibility of biocontrol by *C. procera*, the aim of this study was to evaluate the effect of different *C. procera* aqueous extract concentrations on *P. sojae* and *S. sclerotiorum*.

2. Method

The experiment was carried out at the Laboratory of Phytopathology, State University of Montes Claros, Janaúba *Campus*, Minas Gerais. The Laboratory of Biotechnology and Fungi Genetics, Federal University of Lavras provided *P. sojae* and *S. sclerotiorum* strains. To carry out tests, fungi were grown in PDA medium (Potato, Dextrose, Agar) for seven days at room temperature in continuous dark. *C. procera* leaves were collected in the first morning hours, which were washed under running water and dried on paper towels. Subsequently, 500 grams of leaves were weighed and submitted to disinfection with sodium hypochlorite (1%, 30 seconds), 70% alcohol (30 seconds) and triple washing with distilled water.

2.1 Preparation of Calotropis procera Aqueous Extract

C. procera leaves were cut and crushed in blender with 500 milliliters of distilled water. Then, in laminar flow chamber, the extract was filtered with sterile gauze and filter paper. Then, the liquid was transferred to airtight glass vials and autoclaved at 121 ºC for 20 minutes. The autoclaved extract was deposited in sterile plastic tubes to be centrifuged at 13,000 rpm for 15 minutes. The supernatant was used in tests.

2.2 In vitro effect of Calotropis procera Aqueous Extract on Phomopsis sojae and Sclerotinia sclerotiorum

PDA culture medium was prepared, which was added of *C. procera* aqueous extract (50% v/v) so that the final concentrations in Petri dishes were 5%, 10%, 15% and 20%. In addition, control plates were prepared, that is, PDA medium without the addition of extract. Discs of 3 mm of edge of *P. sojae* and *S. sclerotiorum* cultures grown for seven days were transferred to the center of a 7-cm petri dish.

Discs were kept at room temperature in continuous dark. After 48 hours, the mycelial growth rate was evaluated and after seven days, mycelial growth, fungi sporulation and germination were evaluated. Mycelial growth was measured with the aid of millimeter rule for two days in two perpendicular directions marked at the bottom of each Petri dish. The mycelial growth rate was calculated using formula proposed by Silva et al. (2015) in which there is proportion of mycelial growth subtraction of 48 for 24 hours by subtracting the longest time for the shortest time (48-24). Results were expressed in mm d^{-1}.

2.3 Effect of Calotropis procera Aqueous Extract on the Mycelial Growth, Sporulation and Germination of Phomopsis sojae and Sclerotinia sclerotiorum

After seven days of incubation, the diameter of *P. sojae* and *S. sclerotiorum* colonies submitted to different *C. procera* concentrations was measured with the aid of millimeter rule. Ten milliliters of sterile distilled water plus 0.08% Tween were deposited on plates containing fungal colonies. Spores were placed in suspension with the aid of glass slide. Subsequently, 3 drops of lactophenol were added to the suspension for spore count in Neubauer chamber under optical microscope. To evaluate spore germination, 100 µL of the previously obtained suspension (before applying lactophenol) were transferred to Petri dishes containing agar-water medium and stored at room temperature for 12 hours. After that period, 100 germinated and non-germinated spores were quantified under optical microscope, being considered germinated the spore with germ tube greater than or equal to the spore length.

2.4 Experimental Design and Statistical Analysis

The experiment was carried out in a 2 × 5 factorial scheme, with two fungi and five extract concentrations (0%, 5%, 10%, 15% and 20%), with four replicates. Data were submitted to analysis of variance at 5% probability by the F test. In case of significant interaction ($p < 0.05$), data were unfolded. The averages of the two fungi were submitted to the F test and extract concentrations were submitted to regression analysis, choosing the equation

that best described the data behavior and with the highest determination coefficient (R^2). Analyses were performed using the R software version 3.5 (R Core Team, 2020).

3. Results

The result of the analysis of variance is shown in table 1. Significant interaction was observed between fungi and extract concentrations ($p < 0.05$) for all variables analyzed. All determination coefficients (R^2) of equations were greater than 60%.

Table 1. Summary of the analysis of variance, response variables: Mycelial growth rate, colony diameter, spore germination and sporulation of *P. sojae* and *S. sclerotiorum* as a function of different *C. procera* aqueous extract concentrations

Variation source	Medium Square			
	Mycelial growth rate	Colony diameter	Spore germination	Sporulation
Fungi	0.0038*	1.02*	864.90*	15,444.90*
Concentrations	0.0015*	0.47*	2016.56*	2,333.78
Fungi × Concentrations	0.0026*	0.71*	149.21*	222.83*
Experimental error	0.0000	0.05	23.78*	176.23*
Coefficient of variation (%)	0.43	3.63	5.79	17.87

Note. * Significant at 5% probability by the F test.

Regarding the interaction of fungi within each concentration level, it was found that at concentrations of 0%, 5%, 10% and 15%, the mycelial growth rate of *P. sojae* was lower than that of *S. sclerotiorum* (Table 2). In contrast, at concentration of 20%, the mycelial growth rate of *S. sclerotiorum* was lower (Table 2). Regarding the interaction of different concentrations within each fungus level, quadratic behavior for *P. sojae* was verified. Deriving the equation, minimum point of 9.33% was found, that is, the lowest mycelial growth rate would be verified at this concentration. The mycelial growth rate of *S. sclerotiorum* showed linear behavior, and as the extract concentration increased, the mycelial growth rate decreased (Table 3).

Table 2. Unfolding of the fungal species interaction within each concentration level of the extract of *C. procera*. Variable answer: Mycelial growth rate (mm d^{-1})

Fungi	Concentrations of the aqueous extract of *C. procera*				
	0%	5%	10%	15%	20%
P. sojae	0.3735a	0.3496a	0.3586a	0.3395a	0.3782b
S. sclerotiorum	0.4062b	0.4020b	0.3812b	0.3693b	0.3365a
Minimal significant difference	0.002				

Note. Means followed by the same letter in the column do not differ by the F test at 5% probability.

Table 3. Unfolding of the interaction concentrations of *C. procera* extract within each level of procera fungus species. Variable answer: Mycelial growth rate (mm d^{-1})

Fungi	Equation	R^2
P. sojae	$y = 0.3739^* - 0.0056^*x + 0.0003^*x^2$	64.53%
S. sclerotiorum	$y = 0.4135^* - 0.0034^*x$	93.32%

Note. * Significant at 5% probability by the t test.

The diameter of the *P. sojae* fungal colony was smaller than that of *S. sclerotiorum* when concentrations of 5%, 10% and 15% were used, whereas at concentration of 20%, the extract efficiency against *S. sclerotiorum* was greater (Table 4). Evaluating the diameter within each extract concentration, quadratic behavior was observed for *P. sojae*. Deriving the equation, minimum point of 10.05% was found, a value where the smallest colony diameter would be found (Table 5). On the other hand, the behavior of *S. sclerotiorum* was linear, reducing the diameter as more extract was added to the medium (Table 5).

Table 4. Unfolding of the fungal species interaction within each concentration level of the extract of *C. procera*. Variable answer: Diameter (cm)

Fungi	Concentrations of the aqueous extract of *C. procera*				
	0%	5%	10%	15%	20%
P. sojae	6.27a	5.87a	6.02a	5.70a	6.35a
S. sclerotiorum	6.82b	6.75b	6.75b	6.20b	5.65b
Minimal significant difference	0.32				

Note. Means followed by the same letter in the column do not differ by the F test at 5% probability.

Table 5. Unfolding of the interaction concentrations of *C. procera* extract within each level of procera fungus species. Variable answer: Diameter (cm)

Fungi	Equation	R^2
P. sojae	$y = 6.282143^* - 0.093357^*x + 0.004643^*x^2$	64.12%
S. sclerotiorum	$y = 6.945^* - 0.058^*x$	93.50%

Note. * Significant at 5% probability by the t test.

When assessing the action of the aqueous extract on the sporulation of both fungi, it was observed that at all concentrations used, the effect was greater on *S. sclerotiorum* (Table 6). It was observed that the percentage of action of the extract on *S. sclerotiorum* was 30%, 34%, 42%, 41% and 52% higher than on *P. sojae*. The sporulation of both fungi showed linear behavior (Table 6). As the extract concentration increased by one unit, 2.21 *S. sclerotiorum* sporulation units were reduced (Table 7). For *P. sojae*, as the extract concentration increased by one unit, 2.02 sporulation units were reduced.

Table 6. Unfolding of the fungal species interaction within each concentration level of the extract of *C. procera*. Variable answer: Sporulation ($\times 10^5$)

Fungi	Concentrations of the aqueous extract of *C. procera*				
	0%	5%	10%	15%	20%
P. sojae	107.50b	107.51b	102.25b	83.75b	68.75b
S. sclerotiorum	76.50a	71.00a	58.50a	49.00a	32.50a
Minimal significant difference	15.20				

Note. Means followed by the same letter in the column do not differ by the F test at 5% probability.

Table 7. Unfolding of the interaction concentrations of *C. procera* extract within each level of procera fungus species. Variable answer: Sporulation ($\times 10^5$)

Fungi	Equation	R^2
P. sojae	$y = 114.20^* - 0.25^*x$	87.23%
S. sclerotiorum	$y = 79.55^* - 2.21^* x$	97.53%

Note. * Significant at 5% probability by the t test.

The germination of *P. sojae* and *S. sclerotiorum* spores was negatively affected by the highest extract concentrations. The extract was more efficient in controlling *S. sclerotiorum* at concentrations of 10% and 20% (Table 8). Both *P. sojae* and *S. sclerotiorum* showed linear behavior with respect to spore germination (Table 9). The reduction of the germination of *P. sojae* spores in relation to control was 22% and 32% at concentrations of 15% and 20%. In contrast, at concentration of 10%, the extract showed 25% reduction in the germination of *S. sclerotiorum* spores and at concentrations of 15% and 20%, reductions were even greater, 28% and 44%, respectively.

Table 8. Unfolding of the fungal species interaction within each concentration level of the extract of *C. procera*. Variable answer: Germination

Fungi	Concentrations of the aqueous extract of *C. procera*				
	0%	5%	10%	15%	20%
P. sojae	100a	100a	98.50b	78.00a	68.00b
S. sclerotiorum	100a	94.5a	75.75a	71.75a	56.00a
Minimal significant difference	7.04				

Note. Means followed by the same letter in the column do not differ by the F test at 5% probability.

Table 9. Unfolding of the interaction concentrations of *C. procera* extract within each level of procera fungus species. Variable answer: Germination

Fungi	Equation	R^2
P. sojae	y = 106.10* – 1.72*x	82.17%
S. sclerotiorum	y = 101.75* – 2.21*x	96.46%

Note. * Significant at 5% probability by the t test.

4. Discussion

Results show that at lower concentrations, extracts affected more *P. sojae* than *S. sclerotiorum* in relation to mycelial growth rate. This variable is a distinctive quality that demonstrates the variation in sensitivity from one pathogen to another when under conditions unfavorable to its development (Sittisart et al., 2017; Fourie et al., 2019). When the growth rate is lower, it means that the fungus was more affected by the presence of the extract in the culture medium (Sittisart et al., 2017). The effectiveness of the antifungal action on the *in vitro* development of phytopathogens shows that compounds present in *C. procera* are potential biofungicides (Khanzada et al., 2016).

The diameter of the phytopathogen colony was negatively affected by the application of the aqueous extract. This is consistent with research that showed the efficiency of *C. procera* extract on soil phytopathogens (Etaware, 2019; Ali et al., 2020). Numerous fungal disease management alternatives have been implemented to manage plant pathogenic fungi (Carmona-Hernandez et al., 2019; O'Brien, 2017; Varo et al., 2017). Agrochemicals provide rapid effects on phytopathogens but cause risks to human health and environment (Jabeen et al., 2013; Bello et al., 2020; Etaware, 2019). In our study, *C. procera* has shown significant effect on *P. sojae* and *S. sclerotiorum* even at low concentrations.

Sporulation and germination were also affected by the aqueous extract. This antifungal activity is due to different types of secondary metabolites, such as glycocides, alkaloids and calotropin (Morsy et al., 2016). *C. procera* has a type of protein in its composition that facilitates the permeabilization of the membrane of spores and hyphae of fungi, facilitating its deterioration (Ranjit et al., 2012). *C. procera* inhibited the germination of *Fusarium solani*, *Neurospora* sp. and *Colletotrichum gloeosporioides* (Freitas et al., 2011). Our study demonstrated that *C. procera* can be used for the alternative management of disease-causing fungi in soybeans. Thus, this method can contribute to minimize the risk and danger of toxic fungicides. Future studies with this extract will identify the active compounds responsible for its fungicidal activity.

5. Conclusions

C. procera aqueous extract has fungicidal activity, reducing the mycelial growth rate, colony diameter, sporulation and germination of of *P. sojae* and *S. Sclerotiorum in vitro*.

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Callogenesis and *in vitro* Regeneration of Baru (*Dipteryx alata* Vog.) Esprouts

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Abstract

The aim of this study was to evaluate the effect of different concentrations of 6-benzylaminopurine (BAP) and naphthalene acetic acid (ANA) on calogenesis and regeneration from baru leaf and apex segments. The explants were obtained from baru plants previously established *in vitro* from almonds and cauline apices. The leaf segments were placed in Petri dishes containing MS medium (Murashige & Skoog, 1962) with concentrations (0.0, 2.0, 3.0, 4.0 and 5.0 mg L^{-1}) BAP combined with ANA (0.0 and 2.0 mg L^{-1}). The shoot apices were inoculated in test tubes with the same medium using the concentrations (0.0, 0.5, 1.0 and 1.5 mg L^{-1}) BAP combined (0.0 and 0.1 mg L^{-1}) ANA. After 25 days of inoculation, the percentages of callus and texture in leaf explants and apices were evaluated. The number of shoots was also evaluated by the Scott-Knott test at 5% of probability. The most efficient concentration in the formation of callus in leaves was 3.0 mg L^{-1} BAP + 2.0 mg L^{-1} ANA (68.88%), at apexes the most efficient concentration was 1.0 mg L^{-1} BAP without ANA with 100% calogenesis. The most effective concentration was 1.0 mg L^{-1} BAP without ANA with an average of 1.90 of shoots in relation to the concentrations evaluated.

Keywords: phytoregulators, baru, callus, leaf explants, *Dipteryx alata*

1. Introduction

Cerrado is one of the main Biomas of Brazil, both in area and in biodiversity (Ribeiro & Walter, 2008). Native to this biome, the barueiro (*Dipteryx alata* Vog.) is an economically promising species with multiple uses, such as the exploitation of wood, fruits and seeds (Ratter et al., 2000; Durigan et al., 2011). It is a fruitful arboreal, highly valued for its economic potential, with various possibilities for use in food, forage, forestry, silvopastoral system, landscape and reforestation of degraded areas (Larson, 2014).

Baru has been studied due to lipid and protein properties, as well as the production of bioactive molecules used in the food and pharmaceutical industry. The baru fruit also serves for human consumption, both pulp and baru almond can be used in human nutrition, and the pulp consists mainly of carbohydrates (63%), predominantly starch, insoluble fibers and sugars (Alves et al., 2010). Almond is consumed in nature or toast, has high lipid levels (42%), protein (30%), calcium, phosphorus, manganese and potassium, in addition to iron, zinc, selenium and considerable carbohydrate and fibre level (Souza et al., 2011).

Few reports regarding the micropropagation of *Dipteryx alata* as seed germination *in vitro*, but with inconclusive results (Mamedes & Araújo, 2010). The germination rate of seeds is high, however, the fruits can present seeds with pathogens that will give rise to unhealthy seedlings, since their fruits can remain for a long period in the field. For the purpose of obtaining pathogen-free seedlings, plant tissue culture has been used (Perez, 2004).

Plant tissue cultivation techniques have been effectively used for the clonal propagation and genetic improvement of different crops (Rocha et al., 2012; Sousa et al., 2019), providing higher productive and pathogen-resistant plants, higher vigour and adaptability at environments heterogeneous. Cultivation *in vitro* is an important strategy to solve problems crop using classical genetic improvement and wood biotechnology (Erigs & Schuch, 2005).

Zygotic embryos culture is a very common procedure to regenerate seed embryos that do not germinate under conventional sowing conditions. However, its greatest applicability is through the rescue of immature embryos from developing seeds (Raghavan, 2003).

In vitro embryo cultivation is a promising technique for advancing knowledge about certain species, because, based on such activity, is possible reproduction and embryonic development, dormancy breakdown and plant production (Raghavan, 2003).

Calogenesis is the methodologie more applied to cultivation *in vitro*, also used for production of secondary metabolites and genetic transformation (Santos, 2004). Studies with callus can also serve as initial point for determining necessary conditions of development (Landa et al., 2000). For induction of callus, virtually any part of the plant can be used as an explant. However, it is sought to use those who contain the highest proportion of meristematic tissues or who have the greatest ability to express totipotence where each plant cell had the genetic potential to regenerate a plant (Flores, 2006).

In regeneration of woody plants, usually leaves and internodes developed *in vitro* are explants more used (Pérez-Tornero et al., 2000; Cassana et al., 2007). Significant differences in organogenic capacity *in vitro* are found when varying the type of explant and the nutritional composition of the culture medium. However, most components optimised in the growth medium are the phytoregulators, particularly auxin/cytokinin balance (Erig & Schuch, 2005).

Auxins act in cell division and tissue expansion, while cytokinins are used regularly to stimulate multiple sprouts (auxins and cytokinins act as growth regulators, presenting an important work *in vitro*, cell division and tissue expansion), while cytokinins are regularly used to stimulate multiple sprouts (Morais, 2012).

Among the growth regulators used to induce callus, 2,4-D (2,4 dichlorophenoxyacetic), ANA (1-naphthalene acetic acid), BAP (6-benzylaminopurine) and TDZ (thidiazuron) are the most important. Callus is also obtained in the interaction between auxins and cytokinins.

The physiological effect of each regulator depends on its concentration in the medium, and each part of the plant has a different response to changes in the concentrations of auxins and cytokinins (Pozo et al., 2005). BAP is a cytokinin added to right concentrations to the culture medium and promotes increase in the number buds, leaves and sprouts and increase the production of fresh mass and quality of cultivated plants. ANA is auxin that inhibit the proliferation of sprouts, however, it is widely used in association with BAP, because the interaction between both it can further favor the quality of micropropagated plants (Torres et al., 1998).

Therefore, studies aiming at the regeneration of baru plants *in vitro*, formation of corns, production of multiple shoots aiming at the production of seedlings micropropagated and studies of the production of secondary metabolites may be used by pharmaceutical industry for extracting the active ingredient.

In view of the lack of specific information regarding the *in vitro* propagation of baru, the study aims to evaluate the effect of different concentrations of 6-benzylaminopurine (BAP) and naphthalene acetic acid (ANA), and their combinations, in callogenesis and plant regeneration from leaf segments and stem apex of baru *in vitro*.

2. Materials and Methods

Baru fruits were collected between August and September 2018 in the Campo Grande city, MS.

These fruits were stored at room temperature at the school farm of Catolic University Dom Bosco, Campo Grande, MS.

The seeds were removed from fruits with a hydraulic press, selecting the major size, and the plants *in vitro* were used to remove the leaf segments.

2.1 Callogenesis Procedure From Leaf Segments

Leaves segments (1 cm^2) were placed in Petri dishes containing 30 ml of MS culture medium (Murashige & Skoog, 1962), supplemented with different concentrations of BAP and ANA (Table 1), totaling four treatments with 10 repetitions. Each plot consisted of a Petri dish containing three explants.

Table 1. Treatments used to induce callogenesis from leaf explants baru (*Dipteryx alata* Vog.)

Treatments	BAP and ANA concentrations
T_1	0.0 mg L^{-1} BAP and 0.0 mg L^{-1} ANA
T_2	2.0 mg L^{-1} BAP and 2.0 mg L^{-1} ANA
T_3	3.0 mg L^{-1} BAP and 2.0 mg L^{-1} ANA
T_4	4.0 mg L^{-1} BAP and 2.0 mg L^{-1} ANA
T_5	5.0 mg L^{-1} BAP and 2.0 mg L^{-1} ANA

The MS medium was enriched with 30.0 g L^{-1} sucrose and 7.0 g L^{-1} agar and pH adjusted to 5.8. After inoculation, the material was subjected to seven days of darkness and subsequently transferred to photoperiod of 16 hours and temperature of 27±2 °C under irradiation of 36 μmol of m^{-2} s^{-1}.

After 25 days of inoculation with leaf explants, the average percentage of callus formation and texture was evaluated.

Regarding texture the callus were classified into four types: A) compact (tightly bound cells); B) semi-compact (moderately bound cells); C) friable (loosely bound cells); and D) without reaction.

The callus from leaf explants were transferred to test tubes to obtain regeneration.

Tubes contained 15 ml of MS medium with 2.0 mg L^{-1} BAP combined with 0.5 mg L^{-1} ANA, totaling a treatment with 10 repetitions (each plot consisted of a test tube).

After inoculation, the material was subjected to seven days of darkness and subsequently transferred to photoperiod of 16 hours and temperature of 27±2 °C under irradiation of 36 μmol m^{-2} s^{-1}.

2.2 Stem Callogenesis From Apexes and Regeneration

After disinfestation, the seeds remained embedded for 24 hours in Petri dishes containing sterile distilled water to facilitate the removal of the embryos. After the imbibition period, the embryos were removed with the aid of forceps and scalpel.

The embryos were distributed in tubes containing 110 ml of MS culture medium (Murashige & Skoog, 1962), 30 g L^{-1} sucrose, 100 mg L^{-1} inositol and 7 g L^{-1} of agar with pH adjusted to 5.8±0.1 For one month, the embryos were kept in growth room located in the tissue culture laboratory, under temperature conditions of 25±2 °C under 16 hour photoperiod and irradiation of 36 μmol m^{-2} s^{-1}.

2.3 Stem Regeneration From Apexes

Seedlings germinated *in vitro* were used to evaluate the effect of phytoregulators on callus from stem apexes.

Stem tips containing approximately two leaves, after one month of inoculation were placed in test tubes containing 15 ml of MS medium (Murashige & Skoog, 1962), supplemented with different concentrations of 6-benzylaminopurine (BAP) and naphthalene acetic acid (ANA), (Table 2), totaling seven treatments and 8 repetitions, each experimental unit represented by one test tube, each tube containing one explant.

Table 2. Treatments used to induce callogenesis and stem regeneration from apexes of baru (*Dipteryx alata* Vog.)

Treatments	Concentrations BAP and ANA
T_1	0.0 mg L^{-1} BAP and 0.0 mg L^{-1} ANA
T_2	0.5 mg L^{-1} BAP and 0,0 mg L^{-1} ANA
T_3	0.5 mg L^{-1} BAP and 0.1 mg L^{-1} ANA
T_4	1.0 mg L^{-1} BAP and 0.0 mg L^{-1} ANA
T_5	1.0 mg L^{-1} BAP and 0.1 mg L^{-1} ANA
T_6	1.5 mg L^{-1} BAP and 0.1 mg L^{-1} ANA
T_7	1.5 mg L^{-1} BAP and 0.0 mg L^{-1} ANA

After inoculation of stem tips, they were placed in the dark after 20 days of inoculation of stem tips in medium and evaluated in relation the percentage of callus formation and its texture and the average number of shoots.

Regarding texture, the callus were classified into four types: A) compact (tightly bound cells); B) semi-compact (moderately bound cells); C) friable (loosely bound cells); and D) without reaction.

Average sprouts formed in *Dipteryx alata* Vog. after 20 days of cultivation in medium MS supplemented with different concentrations of phytoregulators BAP and ANA. Shooting averages were compared Scott-Knott test (1974) at 5% probability using the Sisvar® statistical program.

3. Results and Discussion

3.1 Evaluation of Foliar Calogenesis in Segments and Callus Regeneration

Callus can be obtained from fragment of tissues that have the capacity to differentiate into tissues, organs and even embryos (R. Paiva & P. D. O. Paiva, 2001). This process occurs because plant tissues have a high degree of plasticity for cell differentiation (Ikeuchi et al., 2013).

Callus production started on seventh day of cultivation. Concentration 3.0 mg L^{-1} BAP and 2.0 mg L^{-1} ANA, was the one that provided highest number of explants callogenic (68.88%). Concentration 5.0 mg L^{-1} BAP and addition 2.0 mg L^{-1} ANA promoted 24.44% of callus (Table 3). Other treatments evaluated did not provide callus formation (Table 3).

Table 3. Percentage of callogenesis in different concentrations of BAP and ANA in the *in vitro* leaf segments

Concentrations BAP and ANA	Callogenesis (%)
0.0 mg L^{-1} BAP and 0.0 mg L^{-1} ANA	00.00%
2.0 mg L^{-1} BAP and 2.0 mg L^{-1} ANA	00.00%
3.0 mg L^{-1} BAP and 2.0 mg L^{-1} ANA	68.88%
4.0 mg L^{-1} BAP and 2.0 mg L^{-1} ANA	00.00%
5.0 mg L^{-1} BAP and 2.0 mg L^{-1} ANA	24.44%

There was a positive interaction between cytokinin and auxin concentrations in the induction of callus from baru leaf explants. Similar results were observed by Landa et al. (2000), which induced callogenesis in leaf explants of pequizeiro (*Caryocar brasiliense* Camb.) at a concentration 2.0 mg L^{-1} ANA and 1.0 mg L^{-1} BAP, approximately 91% formations of callus were obtained.

There was a positive interaction between the concentrations of calogenesis in baru leaf explants (*Dipteryx alata* Vog.), Rezende et al. (2019) observed better results for callus formation when used 2.5 mg L^{-1} BAP combined with 2.0 mg L^{-1} ANA.

Positive influence of auxin and cytokinin combination was also verified in trials involving calogenesis in several coffee cultivars (Santos et al., 2000). Similarly, in studies of calogenesis in coffee testing different doses of 2.4-D and 2.0 mgL^{-1} kinetin. Maciel (2001) also found greater formation of primary nodular callus with high concentrations of 2.4-D.

The auxins are indispensable for the formation of callus, since they are responsible to start cellular division and control cellular growth and stretching processes (Taiz & Zeiger, 2004). Cytokines are also necessary for plant cell division with positive results in embryogenic callus induction (Pasqual, 2001), confirming callogenesis observed in this experiment was probably favored by the joint action of these phytoregulators.

Santos et al. (2015) used 2.4-D and BAP in calogenesis in *P. carniconectivum* leaf explants, and observed that the effects of 2.4-D and BAP were individually significant in callus induction. 2.4-D induced callus in 77.62% of the explants, while BAP represented 73% in callus inductions.

After fifteenth day of cultivation, it was also observed regarding the texture of the callus, and there was only formation of friable and compact callus. The texture of callus was dependent on the concentrations of BAP and ANA used. Higher percentage of friable callus occurred in concentrations of 3.0 mg L^{-1} BAP combined with 2.0 mg L^{-1} ANA, totaling 35.55 % callus (Figure 1A, Table 4).

However, the highest percentage of compact callus was fifteenth day of cultivation, it was also observed regarding the texture of callus that there was only formation of friable and compact callus. The texture of callus was dependent on the concentrations of BAP and ANA used. Higher percentage of friable callus occurred in concentrations of 3.0 mg L^{-1} BAP combined with 2.0 mg L^{-1} ANA, totaling 35.55 % callus (Figure 1A, Table 4). However, highest percentage of compact callus was observed in the same concentration of 3.0 mg L^{-1} BAP and 2.0 mg L^{-1} ANA totaling 66.66% callus (Figure 1B, Table 4). In Tables 3 and 4, the concentration of 4.0 mg L-1 BAP and 2.0 mg L-1 ANA may have inhibited the induction or formation of primary callus.

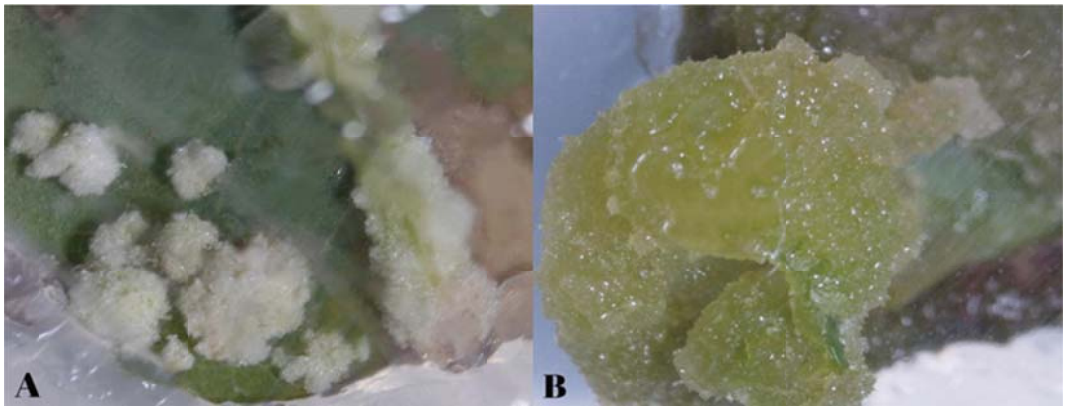

Figure 1. Formation of callus *in vitro* from Baru leaf segments on the thirteenth day of cultivation in MS medium. A: Friable callus; B: Compact callus

Table 4. Percentage of formation of friable and compact callus from *in vitro* Baru leaf segments

Concentrations BAP and ANA	Friable	Compact
0.0 mg L^{-1} BAP and 0.0 mg L^{-1} ANA	00.00%	00.00%
2.0 mg L^{-1} BAP and 2.0 mg L^{-1} ANA	00.00%	00.00%
3.0 mg L^{-1} BAP and 2.0 mg L^{-1} ANA	35.55%	66.66%
4.0 mg L^{-1} BAP and 2.0 mg L^{-1} ANA	00.00%	00.00%
5.0 mg L^{-1} BAP and 2.0 mg L^{-1} ANA	13.33%	6.66%

Different results were obtained in nodal segments of *Pfaffia tuberosa*, where there is a greater proliferation of friable callus in the concentration of 1 mM BAP and 10 mM 2.4-D in MS medium (Flores et al., 2006).

After formation of callus, both in compact and friable callus when they were transferred to the regeneration medium, no regeneration was observed; however, there was formation of more callus and later necrosis of these callus over time. Results different were observed by Dominguez et al. (2006), when the 2.0 mg L^{-1} concentration 2.4-D was used to obtain callus and subsequent regeneration from leaf explants of *Piper auritum* Kunth.

The treatments containing 1 mg L^{-1} BAP with and without addition of ANA and the treatment with 1.5 mg L^{-1} BAP plus 0.1 mg L^{-1} ANA provided 100% calogenesis, while the treatment where only concentration 1.5 mg L^{-1} BAP was added was lower (70%). Other treatments obtained 80 to 90% callus (Table 5).

Table 5. Percentage of stem apexes with callus formation of *Dipteryx alata* Vog., after 20 days of culture in medium MS supplemented with different concentrations of BAP and ANA

Treatment	Calogenesis (%)
0.0 mg L^{-1} BAP and 0.0 mg L^{-1} ANA	0.0%
0.5 mg L^{-1} BAP and 0.0 mg L^{-1} ANA	80%
0.5 mg L^{-1} BAP and 0.1 mg L^{-1} ANA	90%
1.0 mg L^{-1} BAP and 0.0 mg L^{-1} ANA	100%
1.0 mg L^{-1} BAP and 0.1 mg L^{-1} ANA	100%
1.5 mg L^{-1} BAP and 0.1 mg L^{-1} ANA	100%
1.5 mg L^{-1} BAP and 0.0 mg L^{-1} ANA	70%

Callus induction is dependent on an intermediate hormonal balance of auxins, cytokines or both together (Nogueira, 2007). This supply of regulators in the culture medium was sufficient to balance the endogenous content of cytokines in the caulinar apex promoting callus formation.

There was no callus formation without addition of BAP and ANA (Table 2). The results obtained corroborate those of Cordeiro et al. (2004) that observed low calogenesis frequency in *Schizolobium amazonicum* (paricá) in culture medium without growth regulator. However, it is observed that concentration of the BAP regulator was

used, with exception of the treatment 1.5 mg L^{-1} BAP combined with 0.1 mg L^{-1} ANA had a higher incidence of callus (Table 6).

Results found in this work are similar to those found by Machado et al. (2009), who worked with calogenesis induction in *Ananas erectifolius* (Curauá), where the phytoregulator thidiazuron, (group of cytokines) proved efficient inducer of calogenesis.

Formation of callus presented a predominant compact and friable texture, with coloration between green and yellow. Concentrations of 0.5 and 1 mg L^{-1} BAP combined with 0.1 mg L^{-1} ANA promoted highest number of callus compact (90%) (Figure 2B). The highest number of callus friable (100%) was obtained using 1.0 mg L^{-1} BAP and no addition of ANA (Figure 2A, Table 6). The formation of baru callus clusters started from the 20th day of cultivation. In this work we observed a higher occurrence of friable than compact callus. The release of cells, from friable callus, is faster than from compact callus, which favors the regeneration of new plants and the selection of variant biotypes in the improvement programs of species (Pescador et al.; 2000).

Figure 2. *Dipteryx alata* Vog. callus from stem apexes, after 20 days of *in vitro* cultivation.
Left: Friable callus; Right: Compact callus

Table 6. Percentage of friable and compact callus of *Dipteryx alata* Vog. from stem apexes with different dosages of BAP and ANA

Concentrations BAP and ANA	Friable (%)	Compact (%)
0.0 mg L^{-1} BAP and 0.0 mg L^{-1} ANA	0%	0%
0.5 mg L^{-1} BAP and 0.0 mg L^{-1} ANA	60%	20%
0.5 mg L^{-1} BAP and 0.1 mg L^{-1} ANA	10%	90%
1.0 mg L^{-1} BAP and 0.0 mg L^{-1} ANA	100%	0%
1.0 mg L^{-1} BAP and 0.1 mg L^{-1} ANA	10%	90%
1.5 mg L^{-1} BAP and 0.1 mg L^{-1} ANA	40%	30%
1.5 mg L^{-1} BAP and 0.0 mg L^{-1} ANA	50%	50%

Plant regeneration is through tissue culture, which is based on totipotency principle where each plant cell has genetic potential to regenerate a plant (Flores, 2006).

According to the results obtained, it was observed a difference in responses according to concentrations of BAP and ANA used in the number of shoots formed from callus originating from *in vitro* baru stem apexes (Table 7). The production of shoots started from the fifteenth day of cultivation (Figure 3).

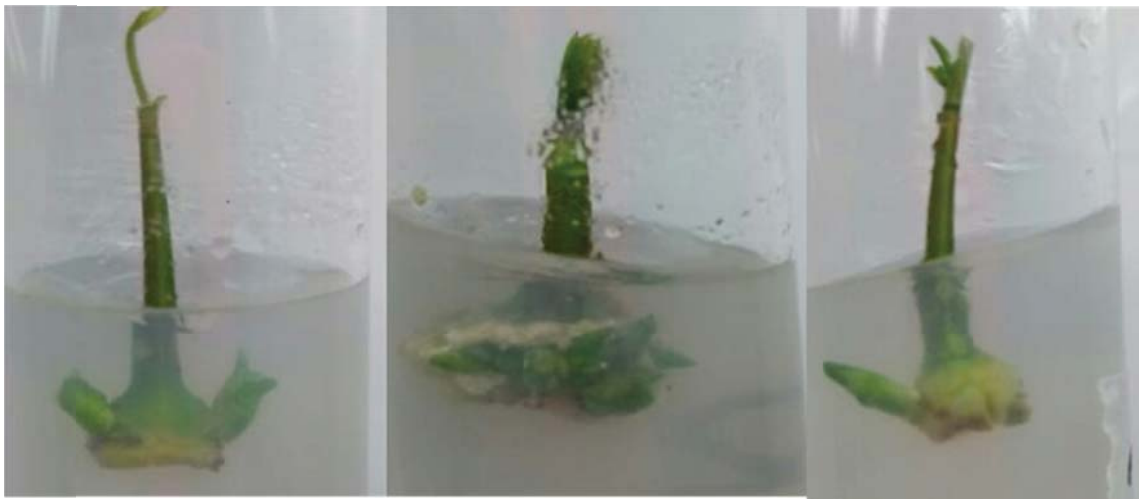

Figure 3. *In vitro* sprouting from stem apexes of baru after 20 days

According to the Table 7, higher numbers of sprouting were obtained in concentrations of 0.5 and 1 mg L^{-1} BAP and no addition of ANA (Table 7). Treatments with 1.5 mg L^{-1} BAP with and without 0.1 mg L^{-1} ANA there was no difference between these concentrations in the number of shoots formed (Table 7). Lower numbers of sprouts were observed in concentrations of 0.5 and 1 mg L^{-1} BAP combined with 0.1 mg L^{-1} ANA (Table 7).

Table 7. Average sprouts formed in *Dipteryx alata* Vog. after 20 days of cultivation in medium MS supplemented with different concentrations of phytoregulators BAP and ANA

Treatment	Shoots
0.0 mg L^{-1} BAP and 0.0 mg L^{-1} ANA	1.00c
0.5 mg L^{-1} BAP and 0.0 mg L^{-1} ANA	1.83[a]
0.5 mg L^{-1} BAP and 0.1 mg L^{-1} ANA	1.00c
1.0 mg L^{-1} BAP and 0.0 mg L^{-1} ANA	1.90[a]
1.0 mg L^{-1} BAP and 0.1 mg L^{-1} ANA	1.10c
1.5 mg L^{-1} BAP and 0.1 mg L^{-1} ANA	1.54b
1.5 mg L^{-1} BAP and 0.0 mg L^{-1} ANA	1.54b
CV (%)	20.02

Note. Averages in the column followed by the same letter do not differ statistically at the 5% probability level by the Scott-Knott test.

Several studies indicate that cytokines are usually suppressed in the culture medium for formation of adventitious sprouts. While auxins are growth regulators that stimulate the formation of early roots in tissues predisposed to rooting (Santos et al., 2003). This fact was observed in this work, where baru explants submitted to BAP concentrations without ANA addition developed higher numbers of sprouts.

According to the results presented, observed that greater sprouting occurred in the absence of ANA. Similar results were observed by Vicente et al. (2009), when working with *Vernonia condensata*, found that concentration of 1.0 mg L^{-1} BAP was the one that provided the best response to the number of sprouts per explant. Work with *Jatropha curcas* using 2.0 mg L^{-1} 6-benzylaminopurine (BAP) without presence of ANA showed greater multiplicity of buds and adventitious shoots (Lopes, 2012). These authors confirmed that the beneficial effect of BAP on sprout multiplication may be related to the influence of this regulator on cell division and on numbness breakdown of axillary yolks, until then inhibited by apical dominance (Brum et al., 2002).

Callus regeneration in *Syzygium aromaticum* using culture medium containing 2.4-D and BAP, positive interaction between auxin and cytokinin was observed, or when culture medium was used, no growth regulator was added (Jain et al., 2001). These authors observed different results from those found in this work, because the use of BAP without ANA in baru explants promoted higher number of sprouts from stem apexes.

4. Conclusions

We concluded that foliar segments of *Dipteryx alata* in medium MS submitted to concentration of 3.0 mg L^{-1} BAP plus 2.0 mg L^{-1} ANA, was the most efficient for callus formation in relation to the evaluated concentrations.

There was no regeneration of callus formed from leaf explants when submitted to concentration of 2.0 mg L^{-1} BAP combined 0.5 mg L^{-1} ANA.

Cellular apices cultivated in MS medium using concentrations of 0.5 and 1.0 mg L^{-1} BAP without addition of ANA and 1.5 mg L^{-1} combined with 0.1 mg L^{-1} ANA, were more efficient for the formation of 100% of callus in relation the evaluated concentrations.

Shoot apexes cultivated in MS medium using concentrations of 0.5 and 1.0 mg L^{-1} BAP without addition of ANA, was most efficient for formation of shoots from callus in relation the evaluated concentrations.

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Risk Economic Viability Focusing on Energy Efficiency in Three Genotypes of Elephant Grass in the Municipality of Alegre, Brazil

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Abstract

The use, on a large scale, of fossil fuels and their derivatives has devastating long-term consequences for mankind. Therefore is an urgent need to seek new alternatives for sustainable energy production. This fact is one of the great challenges to be faced by researchers worldwide. Within this context, the elephant grass has been standing out successfully in the production of biomass for energy purposes. The purpose in this study was to analyze the economic viability of biomass production of three genotypes of elephant grass for energy purposes and to identify the risk by means of the Monte Carlo simulation. The economic indicators were obtained by calculating the Net Present Value (NPV), the Internal Rate of Return (IRR), and the Profitability Index (PI). To determine the degree of uncertainty, analysis of sensitivity was applied. Results indicated viability for all genotypes, especially the Guaçú/I.Z.2, with IRR of 17.79%. Variation in sale price of grass generates a greater impact on profitability, followed by the labor and fertilization costs. The risk of failure was relatively low, with the exception of Capim Cana D'África, 38.16%. Among the three genotypes studied, the G1 genotype (Guaçú/I.Z.2) stood out as the one with the best economic viability.

Keywords: production costs, biomass, *Cenchrus purpureus* (Schumach.) Morrone

1. Introduction

Global energy demand has grown throughout the years, an increase related to industrial production, population growth, new energy using technologies, among other factors. As a result, countries seek to develop new technologies to meet growing demand. The use of alternative renewable sources is a sustainable solution. From this perspective, plant biomass offers economic and environmental advantages since, in addition to generating clean energy, it contributes, through photosynthesis, to the reduction of the greenhouse effect in the reprocessing of CO_2 emissions (Morais et al., 2009).

Elephant grass [*Cenchrus purpureus* (Schumach.) Morrone] has stood out as one of the main crops for this purpose for having a high photosynthetic efficiency and high capacity for production of dry matter and fiber content (Morais et al., 2009; Santos et al., 2014; Mohammed et al., 2015). In addition, it presents high yield, rapid plant growth, high hardiness and resistance to unfavorable climatic conditions, short cycle, and biomass quality attributes (Paterlini et al., 2013).

Trends in the renewable energy market illustrate the strong growth and investments in all market sectors (REN 21, 2013). Brazil is seen as one of the largest agricultural producers and the largest producer and consumer of bioenergy worldwide. Rich biodiversity, availability of land for crops, and adequate climatic conditions are factors that have contributed to high levels of biomass use (Van der Selt, 2011). As such, Brazil is one of the countries with the greatest abundance of renewable energy globally (Borges et al., 2016).

For Ross, Westerfield, and Jordan (2008), a project or investment is worthwhile when it creates value for its owners. For this, it is essential to market what is produced for a price greater than the cost of obtaining it. But for long-term investments (longer than one year), it is also necessary to consider the value of money over time. That is why decision support tools, such as the Net Present Value (NPV), the Internal Rate of Return (IRR), and the Profitability Index (PI), should be used to evaluate the economic viability of an investment.

The relevance of the economic viability analysis of elephant grass relies on the fact that one can measure yield variation with a view to its increase, with a minimum associated cost, optimizing, therefore, the system as a whole. The financial analysis is based on estimates of cash flows, in which the variables involved in the systems are predictable and lead to deterministic financial indicators. In the analysis of economic viability, an attempt is made to identify bottlenecks that generate possibilities to make the success of the project unviable and, thereby, neutralize them, making it possible for its objective to be achieved effectively, not depending on chance, but rather on concrete and effective actions based on the studies performed. A number of authors state that these indexes are used to perform a financial and risk analysis (Aerieira et al., 2008; Lyra et al., 2010; Pereira et al., 2011; Sabbag & Nicodemo, 2011; Ferraz Carvalho et al., 2014).

Given this, the goal of this study was to determine the profitability of dry matter yield of elephant grass biomass for energy purposes, calculating the NPV, IRR, and PI, and evaluating, by sensitivity and risk analyses, the economic viability of planting three genotypes of elephant grass.

2. Method

The experiment was conducted at the facilities of the Instituto Federal do Espírito Santo – campus Alegre, in the south region of the state of Espírito Santo, latitude 20º45′57.9″ S and longitude 41º27′23.93″ W at 126 m altitude. According to the Köppen classification (1948), the climate is of "Cwa" type, *i.e.*, tropical hot and humid, with cold, dry winter. It has an average annual temperature of 23.1 °C and total annual precipitation of 1341 mm (Lima et al., 2008).

The soil in the experimental area is classified as Eutrophic Red Yellow Latosol (Embrapa, 2013). Soil samples were collected at a 0-20 cm depth at the site selected for the experiment, to perform particle size analysis, which resulted in Area I: Sand 80.25%, Silt 2.75%, and Clay 17.0%; in Area II: Sand 78.55%, Silt 2.4%, and Clay 19.05%; and, in Area II: Sand 69.95%, Silt 2.4%, and Clay 27.65%, performed by the Laboratory of Soil Physics of the Center for Agricultural Sciences of the Universidade Federal do Espírito Santo, located in the municipality of Alegre, state of Espírito Santo, Brazil.

This study was conducted with the genetic material from the Germplasm Bank of the Universidade Estadual do Norte Fluminense Darcy Ribeiro, located in the municipality of Campos dos Goytacazes, state of Rio de Janeiro, Brazil. Three genotypes of elephant grass (G1: Guaçú/I.Z.2; G2: Cameroon Piracicaba; and G3: Capim Cana D'África) were selected and tested in a randomized block design with three replicates, in the arrangement of sub-subdivided plots, to verify the potential of genotypes under five levels of nitrogen fertilization and four of phosphate fertilization.

The experimental area was composed of 36 lines of 12 m each and 1.5 m line spacing. Each block comprised 60 experimental units of 2.40 m of linear extension. Stem planting was arranged in line in furrows, joining tip and tail of each other. After that, stems were cut to approximately 50 to 60 cm in size inside the planting furrow and covered with 3 cm of soil.

The experiment was installed in April 2010. Two standardization cuts were made on October 19, 2010 and March 2, 2011, respectively, for climatic factors and failures found in the planting. Three evaluation cuts were performed, the first, on August 29, 2010; the second, on June 25, 2011; and the third evaluation cut was performed on December 22, 2011.

2.1 Economic Viability Analysis

The economic viability analysis was initiated with the construction of cash flows, which enabled to calculate profitability indicators for the activity under consideration. As Noronha (1987) states, the revenues and expenditures of financial resources in a particular project along time represent its cash flow. The effective

revenues form the inflows and the effective expenditures form the outflows, in which the differential is given by net flow (Ponciano et al., 2004).

2.2 Net Present Value and Internal Rate of Return

Net Present Value (NPV) represents the sum of the present value of the expected net cash flows for each period brought to the zero period, at a discount rate (r) equal to the minimum attractiveness rate (Guiducci et al., 2012). Thus, the NPV calculation has the following formula:

$$NPV = \sum_{t-1}^{n} \frac{CF_t}{(1+r)^t} - CF_0 \quad (1)$$

where, CF_0 = initial investment made in the period t = 0; CFt = expected net cash flow in the period t = 0; r = discount rate.

When the value found for the NPV of a project is positive, it is considered the project should be able to generate value for the owners; hence, it should be accepted, because the greater the value found, the greater the possibility of generating wealth. For that reason, when two projects with the same level of risk are analyzed, the rational choice is invariably for the project with the highest NPV. In case the value found is negative, the project must be rejected, because the smaller the NPV, the greater the loss for the owners. If the NPV equals zero, however, its implementation, or not, makes no difference, because the project is unable to generate or destroy wealth (Ross, Westerfield, & Jordan, 2008).

The Internal Rate of Return (IRR) is the discount rate that equals to zero the NPV of an investment opportunity. This means, in practice, the IRR provides the highest acceptable discount rate for the project under analysis to be profitable because it makes the present value of net cash flows to be equal to the initial cost of implementing the project (Lyra et al., 2010; Guiducci et al., 2012; Ferraz Carvalho et al., 2014). As such, mathematically, the IRR is represented following the equation:

$$NPV = \sum_{t-1}^{n} CF_t/(1 + IRR)^t - CF_0 = 0 \quad (2)$$

$$\sum_{t-1}^{n} CF_t/(1 + IRR)^t = CF_0 \quad (3)$$

where, CF_0 = initial investment made in the period t = 0; CF_t = expected net cash flow in period t; IRR = Internal Rate of Return.

Under the IRR method, a project must be executed if the value found is greater than the cost of capital of the project owners, and rejected, if not (Assaf Neto & Lima, 2011). The cost of capital, also called rate of return, discount rate, required return, or opportunity cost, is determined by decision makers themselves (Gitman, 2010). They aim at covering all aspects of return on committed capital during the whole project, which is why they consider factors such as inflation, sources of risk that undermine the capacity of the project to generate revenue (*e.g.*, variation in sale prices and cost structure), and risk of variation in market interest rates (Lemes Júnior, Rigo, & Cherobim, 2010).

All these factors weighted mathematically constitute the cost of capital, which can be understood as a Minimum Attractiveness Rate (MAR) demanded by investors to execute a particular project.

Comparing the recommendations for implementation of projects obtained by the NPV and IRR methods, Gitman (2010) affirms it is only possible to have conflicting classifications if the projects have very different durations from each other; the moment of occurrence of cash flows are at very different points in time line; or if the magnitude of initial investments and/or the cash flows generated in each project are not on the same scale.

Accordingly, this work does not meet any of the conditions mentioned above. All the boundary conditions of the study groups are similar, i.e., the maturation time of the projects (permanence of the study groups in the field), the moments when the cash flows were generated (cuts and sales), and their magnitude (price practiced by the market) are comparable. Therefore, with NPV and IRR methodologies, it can be said that it is possible to obtain a reliable representation of the capacity to generate value from each of the study groups.

The NPV is the most indicated method among academics. Ross et al. (2010) argue that NPV is the best choice for being both a measurement of value added and for the fact that IRR ignores the cash flow scale from different projects. According to Brigham and Ehrhardt (2012), NPV is the best tool to compare investment alternatives, as it is the one that best identifies and informs decision makers about the capacity to create wealth from an investment.

2.3 Profitability Index (PI)

The profitability index, according to Ross et al. (2010), can be represented by the following equation:

$$PI = \frac{\text{Present value of cash flows subsequent to the investment}}{\text{Initial investment}} \quad (4)$$

For Assaf Neto and Lima (2011), PI indicates how much a project returns for each monetary unit in present value. In this way, a profitability index (PI > 1) represents an economically viable project that should be executed because it can generate value for the owners.

Brigham and Ehrhardt (2012) state the greater the PI of a project, the greater its classification. Accordingly to Ross et al. (2010), the PI is subject to the same IRR restrictions, so it must meet the same boundary conditions (projects with cash flows of the same magnitude and time proximity).

For projects independent of each other, the PI, NPV, and IRR will always result in the same acceptance-rejection decisions. For projects independent of each other, the PI, NPV, and IRR will always result in the same acceptance-rejection decisions (Brigham & Ehrhardt, 2012). But in case of conflicting classifications of mutually excluding projects, the order of the projects according to NPV should be chosen (Ross et al., 2010).

2.4 Analysis of Sensitivity

For decision making, it bears in mind that many factors influence the budget of a project. Input and output prices have probabilistic variations. Predicting at what levels prices will be in the future is not an easy task. A number of external and internal factors affect the process. The analysis of sensitivity was applied to estimate the context. Analysis of sensitivity involves choosing the indicator to be sensitized, and determining its expression according to the parameters and variables chosen. In this manner, using a computer program, the results are achieved from the introduction of parameter values in the expression, the simulation is performed, and it is verified in what way and in what proportions these variables affect the result in terms of probabilities (Ponciano et al., 2004).

2.5 Simulation Techniques

To execute the simulations, it was chosen the Monte Carlo simulation method, which is based on the generation of random numbers. The Monte Carlo method is based on the distribution of continuous probability variables. As Triola (1999) says, the distribution function of continuous variables y = F(x) is the sum of the probabilities of all possible values that the variable x can assume until the value of x itself. A continuous random variable (x) can assume any fractional value within a defined value range.

For a discrete random variable, the mathematical expectation is given as:

$$E(x) = x_1P(x_1) + x_2P(x_2) + \ldots + x_{1n}P(x_n) = \sum_{i-1}^{n} x_iP(x_i) \quad (5)$$

The probability density function is given by: $y = e^{-\frac{1}{2}\left(\frac{x-\mu}{\rho}\right)^2} \Big/ \rho\sqrt{2\pi}$, where, μ is the distribution mean and ρ is the standard deviation of the distribution. A standardized normal distribution that has a mean of 0 and the standard deviation equal to 1, with tabulated results, in which z is the standardized normal variable, is determined by:

$$z = \frac{x - \mu}{\sigma} \quad (6)$$

The Monte Carlo simulation was used to prepare a cash flow model. It uses randomized numbers and probability to solve problems and has been widely applied in the literature (Peres et al., 2004; Lima et al., 2007; Mingoti & Glória, 2008; Campos, 2010; Lyra et al., 2010; Sabbag & Costa, 2015).

3. Results and Discussion

The cash flows were structured from the inflows and outflows of resources estimated in data collected in the region of the experiment, with a time frame of five years. It was observed in Table 1, a relationship between costs and the estimated profitability of dry matter, in which the local market pays the average price of R$ 41.82 (forty-one reais and eighty-two cents) per ton of dry matter of elephant grass for energy purposes. The sale price variation of grass is the characteristic that generates the greatest impact on the profitability of the activity, followed by the cost of labor and fertilization.

Table 1. Cost and estimated profitability in dry matter (elephant grass) planted per hectare in the south region of the state of Espírito Santo, municipality of Alegre, 2013

Genotypes	Cost and estimated profitability in dry matter (DM)	
	Cost in R$/ha	Estimated profitability in R$
G1	3196.18	3615.03
G2	3196.18	3504.84
G3	3196.18	3320.00

According to Santos et al. (2014), when evaluating the effect of nitrogen and phosphate fertilization on the dry matter yield in this experiment, it was noticed that the mineral nutrients (nitrogen and phosphorus) did not generate, in general, relevant variations for the dry matter yield process and the average dry matter yield from the biomass of G1, G2, and G3 genotypes was 14.1567; 13.2277; and 11.5592 t ha^{-1}, respectively. In the view of Santos et al. (2016), given the price of a ton of grass and the price of fertilizer, the use of nitrogen fertilizer in the production of elephant grass for energy purposes in the municipality of Alegre is not recommended.

The analysis of sensitivity showed that, in the systems proposed, the estimate of the sale price is the one that has the greatest impact on profitability. Variables such as the growing system, labor, and agricultural inputs were considered important for the success of the project because of their relevance related to the costs of the project. The NPV with a planning horizon was positive for all the discount rates taken into account. To determine the minimum attractiveness rate (MAR) of 9% per year, it was considered the equity and the value close to the average evolution of the Selic rate between 2008 and 2012. In order of importance, the genotypes with the highest values were G1, G2, and G3, respectively. The profitability indexes obtained for the three genotypes analyzed were depicted in Table 2.

Table 2. Net Present Value and Internal Rate of Return related to the planting of elephant grass planting in the region of Alegre, south of the state of Espírito Santo in 2013

Genotypes	Net Present Valueo (R$)						IRR
	1.50%	3.00%	4.50%	6.00%	7.50%	9.00%	
G1	1365.52	1203.04	1049.69	904.81	767.78	638.06	17.79%
G2	1065.69	913.89	770.62	635.26	507.24	386.04	14.39%
G3	629.66	493.75	365.47	244.27	129.65	21.14	9.31%

Considering the estimated values for the IRR(s), all activities are recommended, as they showed values above the MAR equal to 9.0%. Taking into account the order of importance, there was the following information: G1 with IRR = 17.79%; G2 with IRR = 14.39%; and G3 with IRR = 9.31%.

For the G1, G2, and G3 genotypes, an internal rate of return (IRR) of 17.79%, 14.39%, and 9.30% were estimated, respectively. The decreasing behaviors of NPV(s) relative to the respective MAR(s) for G1, G2, and G3 are depicted in Figures 1, 2, and 3.

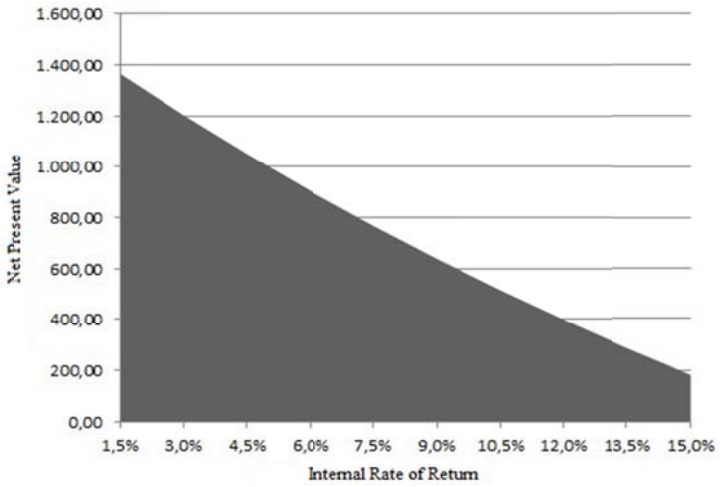

Figure 1. Behavior of NPV with the MAR relative to the G1 genotype

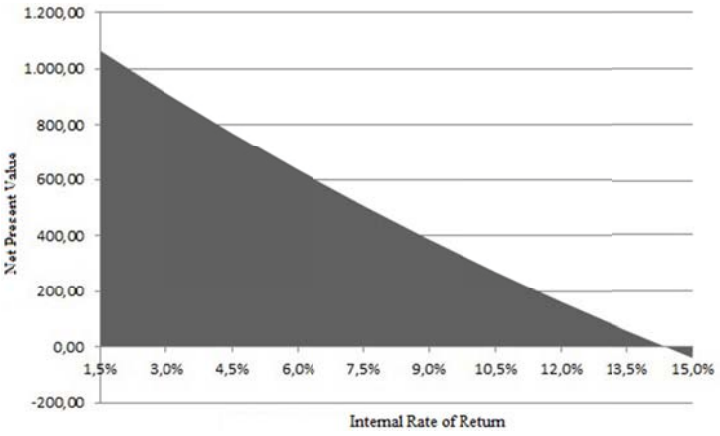

Figure 2. Behavior of NPV with the MAR relative to the G2 genotype

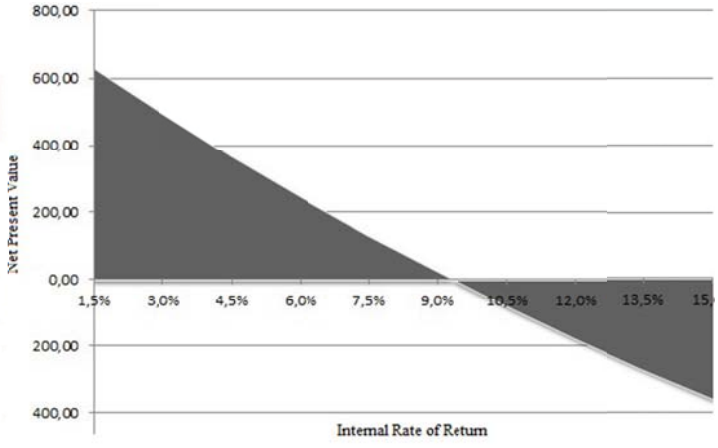

Figure 3. Behavior of NPV with the MAR relative to the G3 genotype

In the Monte Carlo simulation, the estimated NPV value for the G1 genotype was of R$ 418.85, for an estimated IRR of 13.63%; for G2, the value was of R$ 306.66, for an estimated IRR of 11.94%; and, for G3, of R$ 121.82, for an estimated IRR of 9.31%.

The cumulative probability distributions of the net present values for the G2 and G3 genotypes were smaller than for the G1 genotype. The probability estimated for negative net present values (NPV < 0) of the G1 genotype was 1.8%; of G2 was 5.49%; and of G3 genotype was 38.16%. The NPV estimate of the G1 genotype was greater than the NPV estimates of the G2 and G3 genotypes. As such, the risk of failure was relatively low, with the exception of Capim Cana D'Africa (G3), 38.16%.

In Figure 4, it was verified the cumulative probability distribution of the net present value from the Monte Carlo simulation. Since this is a simulation, it should be observed that the cash flows estimated are susceptible to error. Yet, the information collected should not be neglected, as it greatly contributes to decision making.

The PI estimated, in order of importance, were 1.131 for the G1 genotype; 1.096, for G2; and 1.038, for G3. As they are estimates, cash flow projections are subject to errors. The determination of risk estimates was limited since, besides the economic risks, the climatic risks of the region in which the project was conducted were also taken into account.

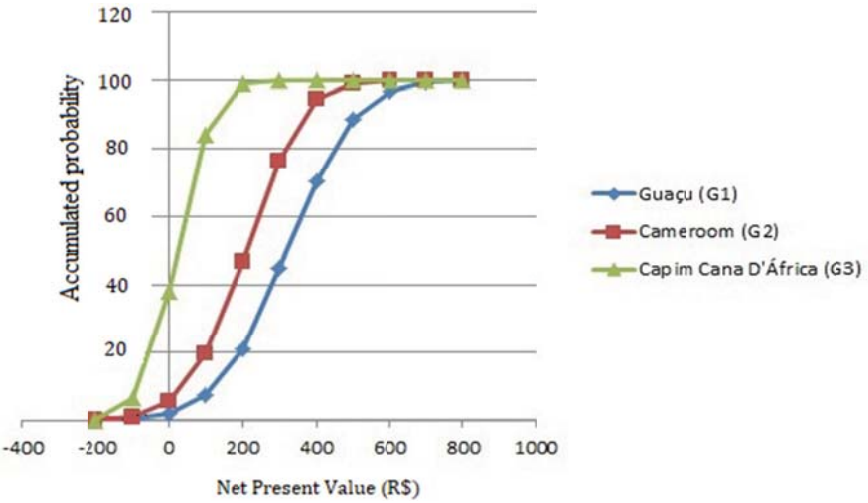

Figure 4. Accumulated probability distribution of the net present value (NPV) obtained in the Monte Carlo Simulation of the Guaçú/I.Z.2 (G1), Cameroon Piracicaba (G2), and Capim Cana D'Africa (G3) genotypes in the municipality of Alegre, state of Espírito Santo, Brazil

4. Conclusions

This study hopes to bring contributions by revealing information that helps decision-making and search for strategies that allow better profitability for the producer.

The genotypes discussed in this work were economically viable and the risk of not reaching success was relatively low.

The risk of not reaching success was relatively low, with the exception of the G3 genotype (Capim Cana D'Africa).

The Guaçú/I.Z.2 genotype was highlighted as the genotype with the best economic viability and the lowest risk of failure for energy purposes in the municipality of Alegre, state of Espírito Santo, Brazil.

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Vertical Distribution of *Euschistus heros* in the Leaf Canopy of Soybean Plants

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Abstract

The occurrence of phytophagous stink bugs in soybeans can result in production losses, if this pest is not properly controlled. Our objective was to study the vertical distribution (intra-plant) of nymphs and adults of *Euschistus heros* (Fabricius, 1798) (Hemiptera: Pentatomidae) in the leaf canopy of soybean plants, during the day. For this, fourteen soybean plants located in one meter of row were evaluated in the field, every three hours between 5 am and 8 pm. The sampled plants were divided into three strata (upper, middle, and lower), where nymphs and adults observed were counted in each stratum and sampling period. The treatments consisted of the three strata of the soybean plants and the different sampling points performed at each time of the day represented the repetitions. An irregular distribution of *E. heros* nymphs and adults was observed in the three studied strata of soybean plants, during the day. At 11 am and 2 pm, when the ambient temperature and solar radiation were highest, both the *E. heros* adults and the nymphs positioned preferentially on the upper stratum of the soybean plants and later migrated to the middle and lower strata when the temperature and solar radiation decreased. This information about the distribution pattern of *E. heros* in the soybean leaf canopy, during the day, provides knowledge for more effective monitoring and control of this pest in soybean crop.

Keywords: behavior, stink bug, *Glycine max*, intra-plant distribution, Pentatomidae

1. Introduction

Brazil is currently the second largest producer of soybean, *Glycine max* (L.) Merrill (Fabaceae: Phaseoleae), in the world and has achieved expanded production and productivity with each new crop. In the 2018/19 season, soybean production in the country was 115,030.1 million tons, with a cultivated area of 35,874.1 million hectares and average productivity of 3.206 kg/ha (CONAB, 2019). This expansion of soybean cultivation can increase the occurrence of phytosanitary problems, such as a higher incidence of pests, diseases, and weeds (Oliveira & Hoffmann-Campo, 2003).

Most of the soybean phytosanitary problems are caused by insects of the Lepidoptera and Hemiptera Orders, which are normally present from the emergence of the plants to the harvest (Sosa-Gómez & Silva, 2010). Among the hemipteran pests, the Neotropical brown stink bug *Euschistus heros* (Fabricius, 1798) (Hemiptera: Pentatomidae) is considered a pest of great importance, as it is the most abundant species among the main soybean pests (Smaniotto & Panizzi, 2015) and can cause quantitative and qualitative damage to the crop, if not properly controlled (Ávila & Grigolli, 2014; Scopel et al. 2016). According to Panizzi et al. (2012), more bioecological studies are required about *E. heros* in the soybean, once its population has increased with each crop. As the population of this pest increases, the damage caused to the soybean crop also becomes more and more significant, and in some cases, may become irreversible and cause significant loss in the production of grains or seeds (Corrêa-Ferreira & Panizzi, 1999).

To better understand and properly control a pest species in a given plant crop, its distribution patterns in plants must be understood (Sevacherian & Stern, 1972). The ideal time and how to apply pesticides in a given plant crop are important information for the successful control of insect pests in crops (Sosa-Gómez & Silva, 2010; Siqueira & Antuniassi, 2011). Results show that the ideal time to control some pest insects in soybean crops are be the coldest times of the day, that is, at dawn and dusk. Rattes & Jakoby (2020) reported that adult's soybean phytophagous stink bugs have a habit of moving to the upper canopy of plants in the morning while at the hottest periods of the day they move to the middle third. Zulin et al. (2018) also found that caterpillars of *Chrysodeixis includens* (Walker, 1858) (Lepidoptera: Noctuidae) were more exposed in the upper strata of soybean plants during the coolest times of the day, such as in the early hours of the morning and at night. Similar work was also carried out by Waite (1980) on soybean for the green stink bug, *Nezara viridula* (Linnaeus, 1758) (Hemiptera, Pentatomidae).

If the most suitable time for the application of the insecticide in a given crop is not determined, the control of insect pests may be compromised. When the pest is in the middle and lower part of the plants, it may be more difficult to reach the target with insecticide application; therefore, the control may be inadequate (Fernandes et al., 2006). This reinforces the importance of conducting studies on the vertical distribution of pest insects in the different crops, to determine their behavior on plants and, consequently, help ensure greater efficiency in both monitoring and management (Wilson et al., 1982).

The objective of this study was to evaluate the vertical distribution pattern (intra-plant) of the stink bug *E. heros* throughout the day in three different strata of soybean plants (upper, middle, and lower), to obtain information that can contribute to more efficient management of this pest in the crop.

2. Material and Methods

2.1 Description of the Area

The study was conducted in a soybean crop sown with the cultivar "Brasmax Poder" in the municipality of Itaporã, in the state of Mato Grosso do Sul, Brazil (latitude 22°16′30″ and Longitude 54°49′0″), during the 2018/2019 season. The population distribution, of nymphs and adults of the brown stink bug, was determined by sampling the upper, middle, and lower part of the soybean plants throughout the day, in the interval between 5 am and 8 pm. Thus, the evaluations were conducted at 5:00 am, 8:00 am, 11:00 am, 2:00 pm, 5:00 pm, and 8:00 pm, on three non-consecutive days (01/31/2019; 02/05/2019, and 02/07/2019). The study started at the stage of complete filling of the grains (R6) and ended at the beginning of the physiological maturation of the soybean crop (R7).

To characterize the three strata of the soybean plants (upper, middle, and lower), a graduated ruler was used to demarcate the maximum height of the plants, this height being divided into three equivalent strata vertically, which corresponded to the upper, middle, and lower strata of the leaf canopy of soybean. At each evaluation point, a graduated ruler was placed vertically beside the plants to delimit each stratum to be sampled, in addition to a 1 m long ruler, horizontally arranged (Figure 1), which marked the lateral evaluation area that encompassed 14 soy plants. Based on the establishment of this sampling unit, the number of *E. heros* nymphs and adults present in the three strata of soybean plants throughout the day was evaluated. The number of insects was counted in the 14 soybean plants present in each sampling unit, considering the three strata (upper, middle, and lower) and 30 different points for each evaluation time. The upper, middle, and lower parts of the plants were considered treatments and repetitions were the different sampling points performed at each time of the day.

Figure 1. Detail of the sampling unit for adults and nymphs of stink bug *Euschistus heros* in the three strata (upper, middle, and lower) of soybean plants

2.2 Analysis of Experimental Data

To analyze the results, the data collected in the three days of evaluation were summed, and the average of insects observed in each stratum and sampling time was calculated. The number of *E. heros* adults and/or nymphs observed in the three strata of soybeans were subjected to analysis of variance and the treatment means compared by the Tukey test at 5% probability. Statistical analyses were performed using the software SAS 9.3. To assist in the interpretation of the results obtained, the average data of temperature, humidity, and solar radiation on the days that the insects were evaluated in soybeans were obtained in the weather station of Embrapa Agropecuária Oeste, municipality of Dourados, in the state of Mato Grosso do Sul, Brazil (22°16′ S and 54°49′ W).

3. Results

The incidence of *E. heros* nymphs was least in the lower stratum of soybean plants during the six sampling periods throughout the day (Figure 2). Likewise, the population of nymphs was less in the upper stratum of soybeans in the first two and last two evaluation times (5 am, 8 am, 5 pm, and 8 pm), being less than the density found in the middle portion of soybeans, however, without differing from that verified in the lower stratum. In these periods of greater abundance of nymphs in the middle stratum, both the average temperature and solar radiation were relatively low and the relative humidity was high (Table 1).

In the 11 am period, the nymph population increased in the upper stratum, reaching the greatest abundance in the 2 pm period, when more than 60% of the nymphs present in the soybean plants were found in this stratum, followed by the middle and lower strata (Figure 2). In these periods with greatest abundance of nymphs in the upper stratum, greater solar radiation in the area and less relative humidity in the environment predominated (Table 1).

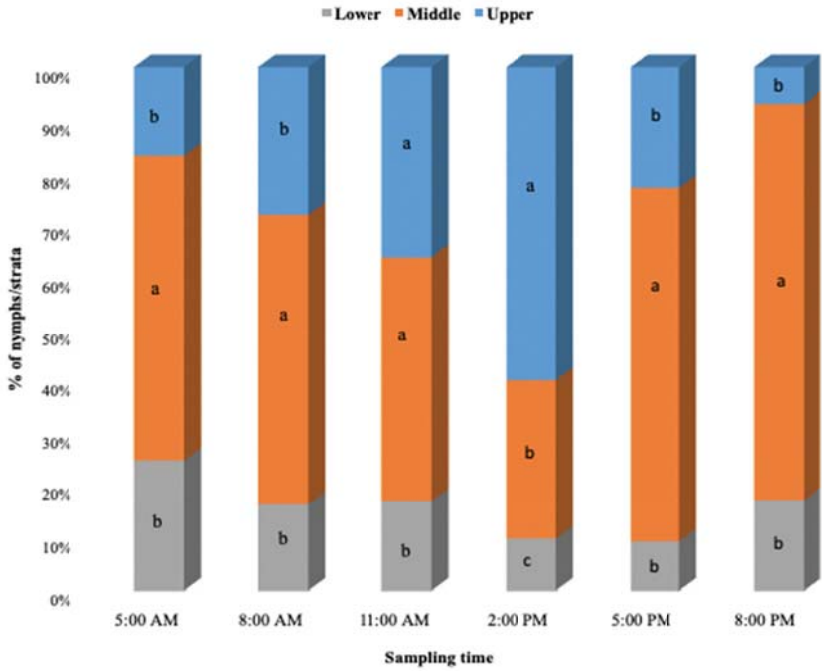

Figure 2. Percentage of nymphs of *Euschistus heros* in the three strata of the soybean plants (lower, middle, and upper), throughout the day, in the 2018/2019 season in Itaporã, MS, Brazil

Table 1. Average temperature (T °C), relative humidity (RH%) and liquid radiation (RN MJ/m^2/day), throughout the day, in the three days of sampling of stink bugs in soybean, in Itaporã, MS, Brazil, 2018/2019

Evaluations	Temperature	Relative humidity	Net Radiation
	---------- °C ----------	---------- % ----------	----- Rn MJ/m²/day ----
5 am	22.33	94.33	0.03
8 am	24.50	86.33	0.62
11 am	28.53	68.33	1.57
2 pm	29.43	64.67	1.02
5 pm	26.63	69.33	0.65
8 pm	25.63	78.67	0.14

The *E. heros* adults presented a distribution pattern in the soybean leaf canopy similar to the nymphs (Figure 3). Likewise, a higher concentration of adults was observed in the upper stratum of the plants at 11 am and mainly at 2 pm, when more than 90% of adults were in this stratum at 2 pm, while in the lower stratum the adult population of the stink bug was practically non-existent in these two hotter periods (Figure 3), when the highest levels of solar radiation, temperature, and lowest values of relative humidity prevailed (Table 1). In the middle stratum of soybeans, a higher concentration of adults was observed at 5 am, 5 pm, and 8 pm hours (Figure 3), times with a predominance of lower temperatures and solar radiation and higher relative humidity (Table 1).

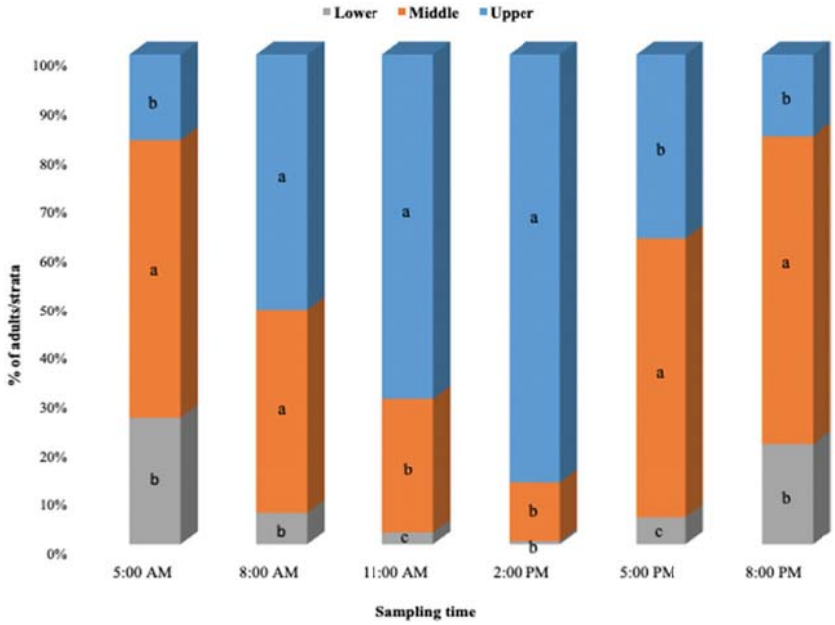

Figure 3. Percentage of *Euschistus heros* adults in the three strata of soybean plants (lower, middle, and upper) throughout the day in the 2018/2019 season in Itaporã, MS, Brazil

When analyzed together the distribution of nymphs + adults *E. heros* in the soybean leaf canopy, throughout the day, it was obvious that at 11 am and 2 pm the insects had a higher concentration in the upper part of the soybean plants, especially compared to the occurrence in the middle and especially lower strata (Figure 4), which is similar to what was observed individually both nymphs and adults (Figures 2 and 3), when the temperature and solar radiation in the environment were high and the relative humidity of the air was low (Table 1). In the cooler times of the day, as observed at 5 am, 5 pm, and 8 pm hours, the adult + nymphs complex has preferred the middle stratum, in comparison to the upper and lower strata (Figure 4), as was also verified separately for the nymphs and adults (Figures 2 and 3).

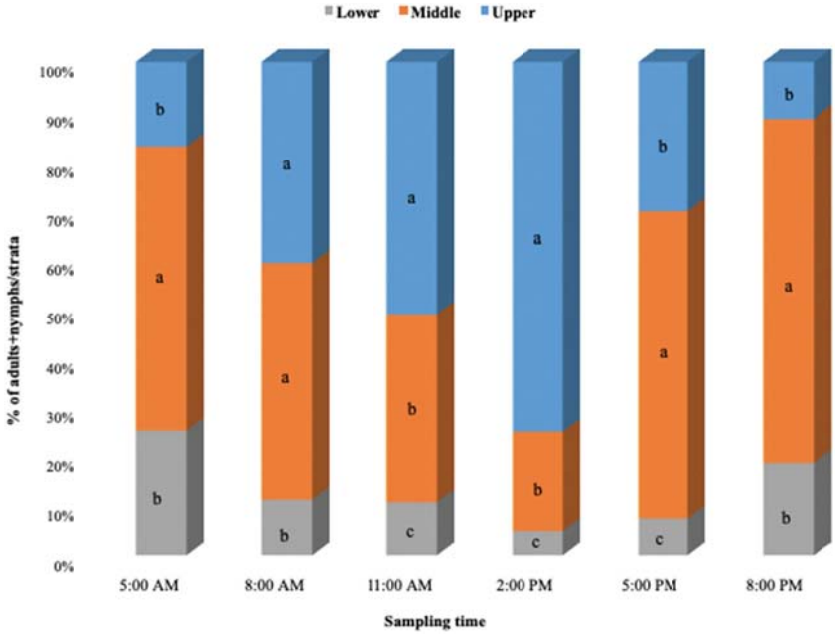

Figure 4. Total percentage of nymphs + adults of the bug *Euschistus heros* in the three strata of the soybean plants (lower, middle, and upper) in the 2018/2019 season in Itaporã, MS, Brazil

4. Discussion

Assessing the intra-plant distribution of the *E. heros* in three soybean cultivars, Engel and Pasini (2019) observed that the damage caused to the pods occurs in all strata of the plants (upper, middle, and lower), although at a higher concentration in the middle and lower thirds. This information was related to the spatial variability of these bugs in soybeans, since through the damages it was possible to verify that the insects were mostly present in the middle and lower strata of the plants, which does not agree with the results for vertical distribution of this species obtained in this work, especially during the hottest times of the day, when stink bugs migrated to the top of the soy canopy.

Waite (1980) studied the behavior of nymphs and adults of *N. viridula* in soybean crop and found greater concentration in the upper part of the plants in the period from 7 to 11 am, corroborating with the results observed in this work for adults of *E. heros* at 11 am (Figure 3). They found that from 11 am onwards, the number of stink bugs in the upper part decreased, returning to the middle and lower strata of the plants, as was verified in this study for adults and nymphs of *E. heros*, which were concentrated in the middle region of the leaf canopy after 2 pm (Figures 2, 3 and 4). It is believed that this behavior of migrating to the lower parts of soybeans is due to the higher temperature and insolation that occurs in the upper part of soybeans, when the insect then seeks the middle and lower part of the plants where temperatures are probably milder. Rattes and Jakoby (2020) argued that phytophagous stink bugs in soybeans, in their adult phase, have the habit of moving to the upper canopy of plants in the morning and in the hottest part of the day they move to the middle third. These reports by Rattes and Jakoby (2020) differ from those observed in this study in that the number of stink bugs in the upper part of soybeans, especially for adults, which increased between 11 am and 2 pm, when the average day temperatures were highest although they migrated for the middle part of soybeans, after these times (Figure 3 and Table 1). Zulin et al. (2018), evaluating the behavior of small and large caterpillars of *C. includens* (Walker, 1858) (Lepidoptera, Noctuidae) in the three strata of soybean plants, observed a higher concentration of caterpillars in the upper stratum of the plant under conditions of milder ambient temperatures. From 10 am, these caterpillars started to move to the middle and lower parts of the soybean plants and later they went to the top of the plants, especially around 8 pm, thus presenting an opposite result from what we observed for *E. heros*, although this was another group of soybean pests.

The fact that *E. heros* adults and nymphs are more exposed at the top of the soybean at times when the temperature and the insolation were highest, may be because these insects are native to the Neotropical Region (Tropical America), thus inducing them to move more intensely at these times (Panizzi & Slansky, 1985). For nymphs, it has already been found that during the grain filling period, their visualization is difficult, since their displacement is less than that of adults, when they stay usually agglomerated in the middle part of the plants (Corrêa-Ferreira et al., 2013).

Based on the results obtained in this study, it can be inferred that chemical control of nymphs and adults of the brown stink bug should be carried out at the hottest times of the day, to increase the control efficiency of this pest, since under these conditions, insects would be more exposed to the action of chemicals applied in spraying the soybean. Nevertheless, these times, especially around 2 pm, are generally not suitable for spraying crops with chemical insecticides, because high temperature and low relative humidity prevail impairs the quality of the application of the products used to control the stink bugs. According to Weber et al. (2017), the deposition of chemicals in the middle and lower strata of the leaf canopy of plants is lower compared to deposition in the upper stratum. Most of the product applied in spraying, whether in high or low volume, is retained in the upper layer, with approximately ten times more coverage in the upper layer than in the lower layer (Boschini et al. 2008; Barbosa et al., 2009). This information reinforces the feasibility of controlling the stink bugs when they are concentrated in the upper part of the soybean plants, between 11 am and 2 pm, as long as there is have adequate temperature and relative humidity conditions for spraying. Dutra et al. (2012) found that the caterpillars of *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), in general have a greater preference for the upper part of cotton plants, during the entire period of their development, a behavior that provides better control of this pest, when insecticide applications are carried out in the crop.

At times of the day when the ambient temperature and solar radiation are higher and the relative humidity of the air is lower, both adults and *E. heros* nymphs prefer to position themselves in the upper layer of soybean plants, moving to the middle and lower strata as the temperature and solar radiation decrease and the relative humidity increases.

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Technical, Field, Economic and Energy Comparison of Cutter Bar Maize Header With Snap Roll Maize Header

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Abstract

In India, most of the maize combine harvester currently being used employs snap roll type header. This type of header is costly, dependent on row spacing of maize crop and causes losses at headlands during turning. Moreover owing to its heavy weight its frequent lifting and downing during harvesting season causes hydraulic leakages in certain sections of combine. Therefore to overcome these problems a new light weight cutter bar Maize header is developed and evaluated for maize crop. The performance evaluation of the cutter bar type maize header is done in a dislodged and a partially lodged (30-40%) maize crop. For lodged crops, the header losses varied from 19.18-26.71% and for dislodged crops it was varied from 5.29-10.15% respectively. The cylinder losses for dislodged crop varied from 2.70-2.86% and for lodged crop it varied from 0.85-2.04%. The mean cleaning efficiency for lodged and dislodged maize crop was found as 88.87% and 90.58% respectively. The grain damage for lodged and dislodged crop was observed as 8.31% and 5.94% respectively. The trash content for lodged and dislodged crop was 2.75 and 3.45% respectively. The performance of snap roll and cutter bar was also done. Total losses with snap roll header were higher as 15.06% and lower for cutter bar as 10.85%. The brokens were higher for cutter bar as 5.94 and lower for snap roll as 3.45%. The trash content was 3.45% for cutter bar header and 2.24% for snap roll header. The total energy input in snap roll header, cutter bar maize header and maize dehusker cum sheller were 2360.05, 1970.90 and 3770.48 MJ/ha respectively.The cost of operatin with cutter bar maize header, snap roll maize header and maize dehusker cum sheller were 53.62 $/ha, 68.73$/ha 187.32 $/ha respectively.

Keywords: combine, snap roll header, cutter bar header, cylinder loss, harvester, header ear loss, maize, crop residues, yield components

1. Introduction

Losses while harvesting can be separated into three categories. Gathering losses that occur at the front of the combine consist of ears (missed or dropped by corn head) and kernels (shelled by the stalk rolls on the corn head). Threshing and separating losses are found on the ground behind the combine. Threshing losses are damaged kernels in the tank and kernels attached to the cobs that were not shelled by the combine rotor or cylinder. Separating losses are loose kernels that were not shaken out of the cobs and husks and were, subsequently, lost over the back of the combine (Humburg et al., 2009; Sumner & Williams, 2009). Mechanisms to gather and cut the crop are located in the header, also called the cutting platform. Slat-type (bat) and pickup reels are commonly used for gathering small grain crops. Pickup reels are used for lodged crops (crops that have fallen over due to heavy rains, winds, etc.), as they have fingers that pick them up for cutting. Proper operation of the reel is critical to minimize header losses that include shatter losses and cutter bar losses. Both these losses are affected by cutter bar height, reel position with respect to the cutter bar, and reel peripheral speed, which is recommended to be about 25-50% faster than the forward speed of the combine (Behroozi-Lar & Mobli, 2006). Grain losses induced from platform of the investigated combine gained 1.29% and losses at the back of the combine was 0.96%. The most amount of damaged grains achieved 10.8% at the speed of 850 rpm for the cylinder (Hassani et al., 2011). The header loss depends on reel rotational speed, ground speed and cutting bar knives. Reel rotational speed and ground speed are mostly efficacious and their losses are 0.5-2% of field yield components (Mazaheri, 1997). Crops with low height couldn't be cut by a cutter, as the seeds drop when they come in contact with the reel. Behroozi-Lar (1995) showed that the reel should be placed in 15-25 cm above the

cutter bar; also, cutting height should be lower than lowest size of crop; furthermore, the reel speed should be adjusted about 1.25-1.50 of ground speed. Mansouri and Minaei (2003) studied the effect of forward speed on header loss and indicated that header loss intensified with an increase in ground speed. A study, using regression analysis model, was performed to estimate and predict the combine header loss at different adjustments of combine header. Three factors were considered as input variables and combine header losses were regarded as output variables. Model showed that the coefficient of determination (r^2) is equal to 0.6292 (Abdi & Jalali, 2013). Qarnar-uz-Zaman et al. (1992) showed that the losses increase with an increase in ground speed. Mostofi et al. (2011) found that the best ground speed for JD 995 was 1.32 km/h. Optimum operating condition of stripper header was obtained with a hood height of 75 cm, header height of 60 cm and rotor speed of 760 rpm. In this condition, the average amount of unstripped loss (header and straw walker) and total loss respectively was 0.54, 1.17 and 1.94% of yield, which indicated considerable decrease of grain losses according to conventional cutter-bar header loss. In all the experiments grain losses decreased with an increase in combine speed.

The results showed that power model was the best model to describe the dependence of the independent variables and the dependent variables. The optimum conditions for the minimum combine header loss (103 kg/ha), reel index, cutting height of crop and horizontal and vertical distances of reel from cutter bar were obtained 1.2 cm, 25 cm, 5 cm, 5 cm respectively (Zareei & Abdollahpour, 2016). Relevant parameters and indicators were established according to the results of the investigations. Fuel consumption was obtained 14.04 l/ha, and 58.97 l/ha for maintaining an efficiency of 24.2 ha/h and an average working of speed 8.0 km/h. The utilization range of investigated harvesters was 70%, with a considerable potential for improvement through better harmonizing of the working regime and the working conditions (Miodragovic & Djevic, 2006). Sensitivity analysis revealed that cylinder speed was the most significant parameter in seed corn harvesting losses (Pishgar-Komleh et al., 2012). Though, harvesting losses cannot be eliminated, yet they can be decreased. Each kilogram of corn (or any other crops) that is saved by careful use of combine, adds to the profit derived from a cultivated hectare (Hanna & Fossen, 1990). Some factors in combine harvester that can reduce corn losses are ground speed, header height, concave, cylinder or rotor speed and cleaning unit (Digman, 2009). So, achieving proper combine setting (ground speed, cylinder speed, cleaning airflow, snapping rolls and spacing between plates) (Hanna, 2008) can help increase combine efficiency, increase grain quality and minimize field losses. Although harvesting losses cannot be removed, they can be reduced to 63 kg ha^{-1} in corn (Hanna & Fossen, 1990). Several studies in this area, such as by Quick (2003) have established a hyperbolic relationship between grain damage and harvested yield for corn combines. He found a certain "sweet spot" where the harvested or bin yield was optimal under the given crop conditions. Corn picker field tests showed that ground speed and snapping roll adjustment are the most important factors determining picking losses (King et al., 1955). Morvaridi et al. (2008) analyzed the effect of ground speed and cylinder speed on corn harvester losses. Results indicated that the effect of cylinder speed was more significant on thresher loss as compared to the ground speed. The maximum total loss (5%) was calculated at ground speed of 2.23 km h^{-1} with the cylinder speed of 550 rpm. The experimental research has substantiated that a variable radius concave with a working plane tilt angle of the oblique concave crossbar equal to 45° would be the rational option for corn ear threshing. In this case, the threshing losses of the grains were minimal (0.03±0.01%), and the maximum share of grains damaged in the threshing unit do not exceed 4% (Pužauskas et al., 2016). Harvest losses were determined for combines harvesting soybean and corn in Brazil. Total soybean combine losses ranged from 47.4 to 260.5 kg/ha (1.2% to 5.5% of yield). The headers were the largest contributors to losses with 31 to 247 kg/ha. Total corn combine losses ranged from 36.2 to 320.6 kg/ha (0.3% to 3.6% of yield). Of this loss, header ear loss accounted for the largest portion with 0 to 237 kg/ha. Shatter losses were the primary cause of losses in the headers. Also, they increased markedly as harvest moistures decreased below 13%. Lodged corn can increase header ear losses as compared with any other source of loss (Paulsen et al., 2014). Threshing, separating and cleaning losses for well-trained combine operators can be very low, rice 0.3%, maize 0.4%, soybeans 0.75-1%, and wheat 1% of yield or less. Losses will go higher when the header is included but in general, rice should be less than 1.25-2.2%, maize less than 1.8%, soybeans less than 3%, and wheat less than 2% of yield in good standing crop (Paulsen et al., 2015). Till present from all the review cited, header plays an important role in minimizing shattering and cutterbar (*i.e.*, header) losses. In most maize predominant areas, only snap roll headers are used in maize harvesting, which is highly dependent on row to row spacing of maize crop leading to higher losses during turnings, improper snap roll spacing and due to operator skill also. Moreover higher cost of snap roll header makes it unfeasible for small and marginal farmers. Therefore a new type of cutter bar type maize header was designed and developed for harvesting of maize crop which cuts the maize plant from a certain height (adjustable) and feeds plant along with cob to the threshing unit of the combine. The maize header was capable of cutting the maize crop, irrespective of the width of the row. The present study was focused to design develop a low cost

cutter bar maize header and investigate its performance for both dislodged and partially lodged maize crop and its economic evaluation with snap roll header and maize dehusker cum sheller in Indian conditions.

2. Method

2.1 Cutter Bar Header Development and Experimental Layout

A first prototype of cutter bar type header with square section reel for maize crop harvester was designed and developed (Figure 1) by the Department of Farm Machinery and Power Engineering, Punjab Agricultural University, Ludhiana, India in collaboration with M/s Nirmal Mechanical Works, Moga, Punjab, India.

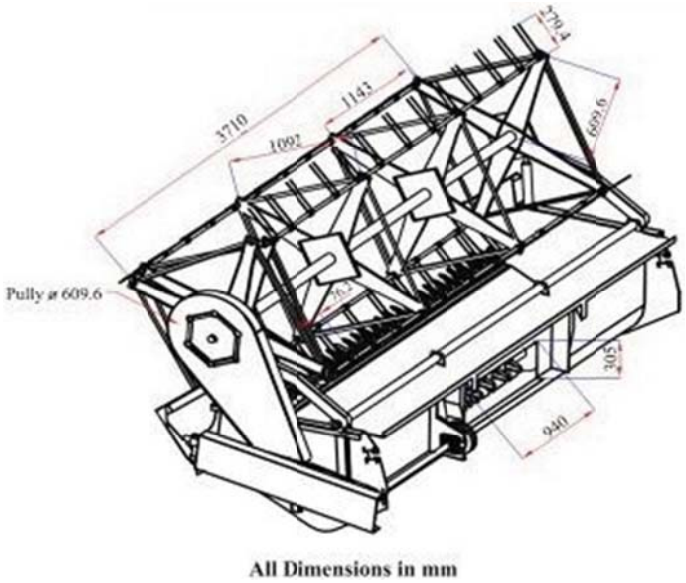

Figure 1. A line diagram of cutter bar type maize header

In this header the conventional cutter bar was used, but with extended fingers. The extended fingers were provided to overcome the thrust coming from maize crop stalk. The fingers were made of mild steel and were tempered to give a greater strength. The reel section was made of mild steel with four sections. The square section reel with spring tines was provided. The reel was made of square section and was bigger in size. The tines were provided in staggered manner, so as, to efficiently collect the cut maize stalk with cobs and minimize the gathering losses. The drive to reel was provided mechanically through belt drive. After initial trials the sizes of driven pulley and pulley driving reel were selected, so as, to reduce the rpm of reel as compared to forward speed of combine. The auger was made of hot rolled sheet. The auger was driven through chain drive and spikes were provided in the middle in a staggered pattern, so as to create a positive feeding action of cut plants towards auger cylinder. The reel was lighter than the conventional snap roll header. The provision was given to adjust height of cut and reel height hydraulically. The crop after being harvested along with maize cobs was conveyed towards auger by a square section reel in a cutter bar header. The detailed specifications of cutter bar type maize header and combine are given in Tables 1 and 2 and a view of cutter bar header and its parts are shown in Figures 2 and 3.

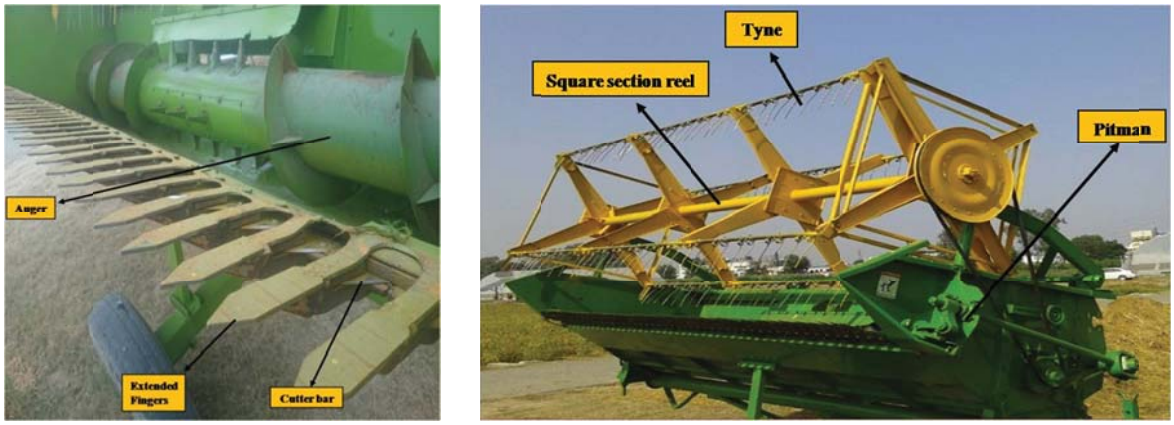

Figure 2. View of developed cutter bar header and extended fingers and auger drum

Figure 3. View of developed cutter bar header and control unit of combine harvester

Table 1. Specifications of cutter bar type maize header

Particulars	Specifications of cutter bar type header	Material of parts
Width of header, mm	3710	-
Total no. of blades on cutter bar	48	High carbon steel
Cutter bar height adjustment	Hydraulic	-
Cutter bar height adjustment range, mm	0-1524	-
Extended Finger length, mm	254	Tempered mild steel
Finger to finger spacing, mm	76.2	-
Reel type	Pick up	Mild steel
		Nylon bush at ends -35 mm
Reel section and side, mm	Square, 1168.40	-
Number of spokes on each square section and length, mm	4, 609.60	Mild steel
Tine length, mm	279.40	Spring wire
Number of tines between two consecutive square sections	10	-
Tine arrangement on consecutive bars	Staggered or alternative.	-
Reel speed adjustment	Mechanical	-
Reel height adjustment	hydraulic	
Reel height adjustment range, mm	38.1-914.4	
Spacing between cutter bar and reel, mm	508	-
Auger window, mm	940 × 305	
Auger diameter, mm	355.60	Hot rolled sheet
Auger bearing at ends		UCF 207 (2)
Auger lugs length, mm	114.30	
Spacing between spokes	203.2	
Driving pulley size, mm	101.60	SG Cast iron
Driven pulley size, mm	609.60	SG Cast iron
Centre to centre spacing between pulleys, mm	1371.60	-
Distance between centre of driven pulley and crop divider edge, mm	609.60	-
Length of platform section, mm	584.2	Mild steel
Length of crop divider edge from cutter bar, mm	685.80	Mild steel
Weight, Kg	1200	-

Table 2. Brief specifications of combine used in field experiments

Different systems of combine	Specifications
Model	Ashok Leyland
Engine Power, Kw (at 2200 RPM)	78.33
No. of Cylinder	Six
Air Cleaner	Combination of Dry & Wet Type
Cooling System	Water Cooled
Clutch	
Type of Clutch	Single, Heavy Duty Dry Clutch
Diameter, mm	310
Transmission	
No. of Gears	3 Forward, 1 Reverse
Forward Gear Speeds, km h^{-1}	L, H
1st Gear	2, 4
2nd Gear	4, 8
3rd Gear	8, 20
Reverse Gear	4, 8
Threshing Mechanism	
Threshing Cylinder Type	Rasp Bar Type
No. of Rasp Bar and Spikes	8, 152
Diameter, mm	606
Width, mm	1250
Speed, rpm	540-1200
Speed Adjustments	By Means of Mechanical Variator
Concave	
Grate Size, mm	35 × 16
Clearance, mm	Front-24, Rear-17
Adjustment	Mechanical
Cleaning Sieves Area, m^2	
Upper Sieve	2.47
Lower Sieve	1.70
Grain Tank, m^3	2.60
Fuel Tank Capacity, ltr	365
No. of Batteries	2
Capacity and Rating of Each, V, Ah	12, 88
Tyre	Size, Ply Rating
Front	18.4/15 × 30, 12/14
Rear	9.00 × 16, 16
Main Dimensions (in working, mm)	
Length	8370
Width	3800
Height	3800
Ground Clearance	340
In Transport, mm	
Length, l, mm	12280
Width, b, mm	3045
Height, h, mm	3800

Maize crop was sown at a research farm of Department of Farm Machinery and Power Engineering and at Farmer's fields during 2014, 2015 and 2016. Maize varieties PMH-2, Pioneer-1844, DKC-9108 was taken for the present study. Maize crop was sown at a recommended spacing of 0.60 m × 0.20 m in experimental plots. The mean stalk height, girth and weight varied between 2.00-2.29 m, 49.23-60.30 mm and 9.58-11.52 Mg ha^{-1} and mean grain yield varied between 6.29-7.02 Mg ha^{-1} [at 21% m.c. (w.b.)] for different experimental plots. The mean cob outer diameter with husk varied between 42.74-44.68 mm. To study the effect of header on various losses, cutter bar type header was tested on the standing and partially lodged maize crop (Figure 3) at the

experimental plots of Department of Farm Machinery and Power Engineering, PAU, Ludhiana and at farmer's fields during 2014, 2015, and 2016. Combine had self propelled engine of 78.33 kW and a rasp bar type threshing cylinder with diameter and width as 606 mm and 1250 mm respectively (Table 3). The overall width of combine was 3800 mm and an effective width of cut was 3000 mm. Effective width of maize header was calculated by measuring the average distance between the centre lines of adjacent picking units multiplied by the number of units. Firstly combine header and combine was checked for all repair and maintenance. Combine was also adjusted according to the cutter bar type maize header. Before operating the combine harvester in the field, by collecting samples from different locations, total grain yield was recorded in the field. The pre-harvest losses were also measured. Combine was operated along the longer length of the field. After operating the combine in the field, various data samples were collected to calculate the gathering losses, unthreshed losses and grain quality parameters like cleaning efficiency, grain damage, trash content etc. In this header ears were fed into the threshing unit along with the stalk. The crop and operational parameters like moisture content, reel operational rpm, threshing cylinder rpm, combine forward speed width of cut, height of cut, fuel consumption etc. were measured.

Figure 4. A view of experimental plot of lodged maize crop

Figure 5. A view of maize harvesting with combine equipped with snap roll maize header

Figure 6. Snap roll

Figure 7. Chain conveyor

Figure 8. A view of snap roll type maize header

The 6 row snap roll maize header combine (Figure 8) was used for comparison with cutter bar maize header. The maize harvester with snap roll header was operated in maize crop (Figure 5). The view of field with lodged crop is shown in Figure 4 and snap roll and chain conveyor are shown in Figures 6 and 7. Specifications of snap roll maize header used are given in Table 4.

Table 4. Specifications of snap roll type maize header

Particulars	Specifications of snap roll type header
Width of header, m	3.60
Spacing between crop dividers, mm	600
No. of rows	6
Total no. of blades	12
Spacing between cutter bar and reel/Number of cutter blade per snap roll	4 (fitted 90º to each other)
Auger window, mm	1295 × 330
Weight, Kg	1,860

2.2 Equipment and Measurement

2.2.1 Digital Moisture Meter

The specifications of digital moisture meter used to measure grain moisture content are given in Table 5.

Table 5. Specifications of digital moisture meter (direct reading type) (Model No. AG-72)

Range	3.5%-40%
Principle	Resistance Measurement
Accuracy	±0.2%
Display	Three Seven Segment Displays
Weight	9.5 Kg Approx. Without Accessories
Dimensions	30 × 17 × 26 cms Approx.
Power	Six 9V Dry Cells or 230 volts, 50 Hz AC Through Adopter
Temperature	Automatic

Stop watch was used to measure the forward speed of the combine. Measuring tape was used to measure the dimensions of the field. For different losses, a cloth was used to collect the samples from different locations of the field. Weighing balance was used to measure the weight of threshed grain, unthreshed grain and straw of maize crop.

2.2.2 Weighing Machine

The weighing of grains and biomass was done using an electronic weighing balance. For measuring plot dimension a tape was used. The unthreshed cobs and shattered ears from the field were handpicked.

For measuring the header losses, cobs were collected from the field in 3 × 3 m^2 area at the different locations in the field. Grains were then collected from the cobs and were weighed to calculate the loss.

2.2.3 Field Operational Parameters Forward Speed, Reel rpm and Width of Cut

At different moisture contents maize crop was harvested. The operational speed of machine was calculated as:

$$v = 3.6 \times S/T \quad (1)$$

where, v is operational speed, km h^{-1}; S is distance covered in meter and T is time taken in seconds.

Tachometer (Line seiki made) was used to measure operational reel rpm during field operation (Table 6 and Figure 9).

Table 6. Specifications of tachometer

Model	TM-5000
Make	Line seiki
Measuring Range	6.0-99999.9 r/min
Resolution	0.1 r/min
Accuracy	±0.01%±1 digit r/min, m/min (for other units, the conversion accuracy is ±0.05%±1 digit)
Sampling Time	1.0-10.0 seconds
Display	Display: 6 digits, 7 segment LCD Battery alarm: ▬ mark Reflective light: (((mark Display unit: r/min
Auto Power-Off	After 3 min from last measurement or key operation
Data Hold Time	Measurement data: until the next data is defined -
Measuring Method	Non-contact measurement using the main unit or with remote sensor (use with reflective tape) Contact measurement using the in-contact adaptor (use with rubber tip, surface speed wheel)
Measuring Distance	50300 mm (using reflective tape)
Power Supply	4 pcs. of AAA alkaline battery (continuous measurement of 20 hrs.)
Operating Temp.	5-40 °C (Non-freezing)
Storage Temp.	-10-60 °C (Non-freezing)
Storage Humidity	35-85% RH (Non-condensing)
Dimension/Wight	122 (H) × 58 (46) (W) × 28 (D) mm/Approx.·130 g (including batteries)

Figure 9. Measurement of reel rpm with tachometer at various engine speeds

Note. The width of cut was measured using a measuring tape.

2.3 Estimation of Field Capacity and Various Losses Measurement

The effective field capacity was determined by measuring all the time elements involved while harvesting. The total time was categorized into the productive and non-productive time. The productive time is the actual time used for harvesting the grains while the non-productive time consisted of the turning time, repair and adjustment time and other time losses. The area covered divided by the total time gave the effective field capacity. The effective field capacity of combine was calculated using the following formula (Kepner et al., 1978):

$$C = \frac{SW}{10} \times \frac{E_f}{100} \quad (2)$$

where, C: effective field capacity, ha h^{-1}; S: speed of travel, km h^{-1}; W: rated width of implement, m; Ef: Field efficiency, in percent.

$$E_f = 100 \frac{T_0}{T_e + T_h + T_a} \quad (3)$$

where, T_0: theoretical time per hectare (per acre); T_e: effective operating time = $T_0 \times 100/K$; K: percent of implement width actually utilized; T_h: time lost per acre due to interruptions that are not proportional to area. At least part of T_h usually tends to be proportional to T_e; T_a: time lost per acre due to interruptions that tend to be proportional to area.

2.3.1 Estimation of Fuel Consumption

Before starting the test, the engine's fuel tank was completely filled. The quantity of fuel required to fill the tank after harvesting the test field was measured using a 1 l graduated cylinder. Thus, the fuel consumed during the test was determined.

$$F = L/A \quad (4)$$

where, F is the fuel consumption in l ha^{-1}; A is the area harvested in ha; and L is the quantity of fuel required to fill the tank after harvesting the test field in l.

2.3.2 Caluclulations of Various Losses and Grain Quality Parameters in Combine Operation

(1) Header Ear Loss

For measuring header losses, data for fallen cobs and kernels in front of machine where the separator had not yet passed. The combine was backed off by a distance equal to length of combine. Loose kernels, broken and whole cobs were gathered from this front area (w × l). These were gathered to calculate the header losses. The header ear losses were calculated as

$$Header\ ear\ loss,\ (\%) = \frac{Weight\ of\ grains\ [Loose\ and\ from\ fallen\ cobs\ (kg)]}{Total\ grain\ yield\ (kg)} \times 100\% \quad (5)$$

(2) Cylinder Loss and Grain Quality Parameters

For measuring cylinder loss kernels still attached to the threshed cobs were collected from 1/100 acre area and weighed. The small kernels at the butt and tip end of cobs were not taken.

The loss of grains and ears which are left unthreshed by the combine over a unit area.

$$Cylinder\ loss,\ (\%) = \frac{Weight\ of\ grains, unthreshed\ left\ on\ ground\ (kg)}{Total\ grain\ yield\ (kg)} \times 100\% \quad (6)$$

After the operation, samples weighing 200 g of grains were collected from the grain tank of the combine. These samples were then cleaned to get the trash content, broken grains and clean grains.

$$Grain\ damage,\ (\%) = \frac{Weight\ of\ broken\ grains\ (kg)}{Weight\ of\ original\ sample\ (kg)} \times 100\% \quad (7)$$

$$Cleaning\ efficiency,\ (\%) = \frac{Weight\ of\ clean\ grains\ (kg)}{Weight\ of\ original\ sample\ (kg)} \times 100\% \quad (8)$$

$$Trash\ content,\ (\%) = \frac{Weight\ of\ trash\ (kg)}{Weight\ of\ original\ sample\ (kg)} \times 100\% \quad (9)$$

2.4 Energy Calculations

Following equations were used for energy calculations in maize combine harvester with various headers:

Human energy consumption (MJ/ha) = No. of human labour used × Time (h) × Human energy equivalent (MJ/h)/Area covered (ha);

Fuel energy consumption (MJ/ha) = Fuel consumption (l/h) × Fuel energy equivalents (MJ/l)/Effective field capacity (ha/h);

Enrgy embodied in machinery (MJ/ha) = Weight of specific machine (kg) × Energy equivalent of machinery (MJ/kg)/Wear out life of machine (h) × Effective field capacity (ha/h).

2.5 Economics

The economics of maize crop harvesting was also calculated for cutter bar header in comparison to the snap roll maize header and maize dehusker cum sheller.

2.6 Statistical Analysis

The software CPCS1 was used for statistical analysis of various treatments of field experiments conducted in present study. The maize combines were operated with snap roll header and cutter bar maize header and experiments were replicated and then statistical analysis was done.

3. Results and Discussion

The maize yield, grain moisture content and pre-harvest losses were measured prior to combine operation and are shown in Tables 7 and 8. The cutter bar maize header was operated in both dislodged and partially lodged maize crop (Figures 10 and 11). The dislodged crop variety had mean grain yield of 7.0 Mg ha^{-1}, whereas the mean grain yield of lodged crop was 3.45 Mg ha^{-1}. The combine harvester with both headers during field operation is shown in Figure 12.

Figure 10. A view of lodged maize harvesting with combine equipped with cutter bar type maize header

Figure 11. A view of dislodged maize harvesting with combine equipped with cutter type maize header

Figure 12. Another view of cutter bar and snap roll maize header during field operation

Table 7. Pre-harvest losses for maize crop

Pre-harvest loss for maize crop (%)				
		1	2	3
Cutter bar Type	Pre-harvest collected grains wt. per ha (kg)	84.87	164.91	134.21
	Total grain weight per ha (kg)	3450	3450	3450
	Pre-harvest Loss (%)	2.46	4.78	3.89
	Mean±S.E	**3.71±0.48**		

Table 8. Operational parameters for cutter bar type maize combine

Crop and operational parameters	Dislodged crop		Lodged crop		CD (5%)
	Range	Mean±S.E.	Range	Mean±S.E.	
Maize crop m.c. (% w.b.)	13.67-14.23	13.97	13.67-14.23	13.97	-
Gear used	1st low-1st medium	1st low-1st medium	1st low-1st medium	1st low-1st medium	-
Engine rpm	1600-1700	-	1600-1700	-	-
Forward speed, km/hr	2.10-2.50	2.33±0.09	2.10-2.45	2.28±0.07	NS
Width of cut, m	3.65	3.65	3.65	3.65	-
Field capacity, ha/h	0.32-0.40	0.36±0.02	0.44-0.53	0.48±0.02	0.0992558
Fuel consumption, l/h	8.0-11.0	11.25±0.35	8.0-9.0	8.30±0.07	1.32605
Fuel consumption, l/ha	20.0-27.5	28.12±0.28	15.70-18.45	17.51±0.70	2.73732
Height of cut, m	0.32-0.45	0.36	Close to ground	-	
Threshing cylinder rpm	600-700	-	600-700	-	-
Reel/snap roll rpm	35-40	-	40-42	-	-

The view of field after combine operation is shown in Figure 13 and various parameters measured are shown in Table 8.

Figure 13. A view of field after the combine harvester operation and forward speed measurement

The height of cut varied between 0.32-0.45 m in case of dislodged crop. In case of lodged crop, header was kept near the ground. The fuel consumption was higher in dislodged crop (28.12 l/ha) as compared to the lodged crop (17.51 l/ha). The reason for higher fuel consumption was continuous maize stalk feeding to combine in dislodged crop. Due to this reason, the mean field capacity was also higher (0.48 ha/h) in lodged crop. The thresher rpm and reel rpm for dislodged crop varied between 600-700 and 35-40 respectively as shown in Table 6. The reel rpm were kept higher in lodged crop so as to pick the maize stalks.

The mean header ear loss was 8.05% in dislodged crop, whereas it was 23.68%in lodged crop (Table 9). Though the cutter bar type maize header was adjusted to nearly horizontal position, yet the lodged crop was not picked completely. Cutter bar header passed over fully lodged crop without picking the cobs, which lead to a higher gathering losses. Cutter bar header managed to pick cobs from those plants which though lodged yet had cobs positioned at some height from ground. The cylinder losses were bit higher (2.81%) in dislodged crop as compared to lodged crop (1.60%). The higher cylinder loss in dislodged crop may be attributed to the fact as though the height was adjusted between 0.32-.45 m still due to continuous feeding of non grain matter as compared with lodged crop. Similar reason could be attributed to higher unthreshed losses in dislodged crop as compared to lodged crop. However, cleaning efficiency was higher (90.58%) in dislodged crop and grain damage was more (8.31%) in lodged crop. The damage was due to non uniform feeding of crop to threshing cylinder which led to more impacts on cobs and grain damage. The trash content for dislodged and lodged crop were 3.46% and 2.75% respectively. However, the effect of position of crop on cleaning efficiency, grain damage and trash content were found to be statistically non significant.

Table 9. Maize grain harvested with cutter bar type maize combine

	Dislodged				Lodged				CD (5%)
	1	2	3	Mean±S.E.	1	2	3	Mean±S.E.	
Grain yield per ha (kg)	7000	7000	7000	7000.00	3450	3450	3450	3450.00	0.358469-05
Shattered grains weight per ha (kg)	710.42	370.15	610.36	563.64±74.48	867.33	661.71	921.50	816.85±59.71	NS
Header ear loss (%)	10.15	5.29	8.72	8.05±1.06	25.14	19.18	26.71	23.68±1.73	7.52263
Unthreshed grains weight per ha (kg)	200.33	188.88	200.10	196.44±2.91	66.24	70.38	29.32	55.31±10.00	37.7158
Cylinder loss (%)	2.86	2.70	2.86	2.81±0.04	1.92	2.04	0.85	1.60±0.29	1.06138
Cleaning efficiency (%)	90.36	88.28	93.10	90.58±0.97	90.34	89.18	87.10	88.87±0.68	NS
Grain damage (%)	5.72	7.42	4.70	5.95±0.57	7.62	8.12	9.20	8.31±0.34	NS
Trash content (%)	3.90	4.28	2.19	3.46±0.49	1.99	2.65	3.62	2.75±0.33	NS

The performance of snap roll header was compared with cutter bar header and operational parameters were measured for both and are shown in Table 10.

Table 10. Operational parameters for cutter bar and snap roll type maize header

	Cutter bar type header		Snap roll type header	
	Range	Mean	Range	Mean
Forward speed , km/hr	2.10-2.50	2.33	1.50-1.70	1.60
Width of cut, m	3.65	3.65	3.60	3.60
Field capacity, ha/h	0.32-0.40	0.36	0.20-0.50	0.28
Fuel consumption, l/h	8-11	11.25	7-10	8.50
Fuel consumption, l/ha	25.00-27.50	31.25	20-35	30.35
Height of cut, m	0.32-0.45	0.36	0.40-0.45	0.42
Threshing cylinder rpm	600-700	-	600-700	-
Reel/snap roll rpm	35-40	-	450-500	-

The performance of snap roll and cutter bar header with maize combine was also done and are shown in Tables 11 and 12. Total losses with snap roll header were higher as 15.06% and lower for cutter bar as 10.85%. The brokens were higher for cutter bar as 5.94 and lower for snap roll as 3.45%. The trash content was 3.45% for cutter bar header and 2.24% for snap roll header. The higher trash and broken for cutter bar may be attributed to higher non grain portion as compared to cutter bar header.

Table 11. Quality of maize grain harvested with cutter bar and snap roll maize header.

Maize grain threshing quality parameters	Cutter bar Type header				Snap Roll Type header				CD (5%)
	1	2	3	Mean±S.E.	1	2	3	Mean±S.E.	
Cleaning Efficiency (%)	90.36	88.28	93.10	90.58±1.50	94.85	94.76	94.09	94.76±0.24	3.93194
Broken loss (%)	5.72	7.42	4.70	5.94±0.70	3.05	2.68	3.26	3.00±0.17	2.25258
Trash content (%)	3.90	4.28	2.19	3.45±0.64	2.41	2.45	1.87	2.24±0.19	NS

Table 12. Total field losses with cutter bar and snap roll maize header

Header and cylinder losses	Cutter bar Type header				Snap Roll Type				CD (5%)
	1	2	3	Mean±S.E.	1	2	3	Mean±S.E.	
Total weight of lost grains per ha (kg)	910.33	558.88	810.00	759.74	1373.33	813.34	976.67	1054.34	-
Total weight of grains per ha (kg)	7000	7000	7000	7000	7000	7000	7000	7000	-
Total Loss (%)	13.00	7.98	11.57	10.85±1.49	19.61	11.61	13.95	15.06±2.37	NS

The economic analysis of cutter bar header was done with snap roll type maize header and conventional maize dehusker cum sheller, which is shown in Table 13. The saving in cost and time with cutter bar type header was 77.77% and 85.42% as compared to maize dehusker cum sheller. The saving in cost and time with snap roll maize header was 71.72% and 83.68% as compared to maize dehusker cum sheller.

Table 13. Economics of cutter bar type maize header, snap roll header and maize dehusker cum sheller

	Tractor 45-50HP	Maize dehusker cum sheller	snap roll	cutter bar
New cost, P	550000	120000	500000	180000
Life (yrs), L	15	10	10	10
Avg. use/yr (h)	700	200	700	300
Rate of interest (%), i	12	12	12	12
Field capacity, ha/h	Of implement	0.17	0.28	0.36
Salvage value, S = 10% of P	55000	12000	50000	18000
Annual Fixed Charges				
Depreciation (Rs/yr)	33000	10800	45000	16200
Interest cost (Rs/yr)	36300	7920	33000	11880
Taxes, insurance and shelter (Rs/yr) = 2% of P	11000	2400	10000	3600
Total fixed costs (Rs/yr)	80300	21120	88000	31680
Total fixed costs (Rs/h)	114.71	105.60	125.71	105.60
Variable Costs				
Fuel required (l/h) (depend on implement	0	8	10	11.25
Labour required with machine	1	1	5	5
Labour cost (Rs/h)	40	31.25	31.25	20
Repair & maintenance (Rs/h)	39.29	30.00	35.71	30.00
Fuel cost (Rs/h) at rs68/l	0	544	680	765
Cost of lubricants (Rs/h) = 20% of fuel cost	0	108.8	136	153
Labor cost (Rs/h)	40	31.25	156.25	100
Total variable cost (Rs/h)	79.29	714.05	1007.96	1048.00
Total Costs				
Total cost (fixed + variable) (Rs/h)	194.00	819.65	1133.68	1153.60
Total cost, Rs/ha including tractor		5962.65	4741.71	3743.33
Labour required off machine operation, man (h/ha)		250	10	10
Grand Total machine Cost, Rs/ha		**13775.15**	**5054.21**	**3943.33**

Particulars	Cutter bar maize header	Snap roll maize header	Maize dehusker cum sheller
New cost (Rs unit^{-1}), P	180000	500000	120000
USD ($ unit^{-1})	2803.55	7787.63	1869.03
Cost of operation, Rs/ha	3943.33	5054.21	13775.15
USD$/ha*	53.62 $	68.73 $	187.32 $
Field capacity, ha/h	0.36	0.28	0.17
Man-h involved per ha	42.00	47.00	288.00
Saving in cost as compared to maize dehusker cum sheller, %	77.77	71.72	-
Saving in time as compared to maize dehusker cum sheller, %	85.42	83.68	-
Saving in cost and time as compared to snap roll header	21.98, 10.64%		
Weight, kg	1200	1860	815
Human energy consumption, MJ/ha	82.32	92.12	564.48
Fuel Energy consumption, MJ/ha	1759.69	2011.07	2649.88
Energy embodied in machinery, MJ/ha	128.89	256.86	556.12
Energy embodied in machinery, MJ/ha	1970.90	2360.05	3770.48

Note. * 1USD = 73.54 INR.

4. Summary

A new type of cutter bar type maize header was designed and developed for harvesting of maize crop which cuts the maize plant from a certain height and feeds plant along with cob to the threshing unit of the combine. Height of cut was adjustable. The maize header was capable of cutting of maize crop irrespective of maize crop row width. The pre-harvest losses varied from 84.87-164.91 kg/ha. For lodged crops the gathering losses varied from 19.18-26.71% and for unlodged crops varied from 5.28-10.14% respectively. The higher gathering losses in lodged crop may be attributed to fact that header could not pick the lodged crop whereas in unlodged crop the header picked cobs from maize plant efficiently. The cylinder losses for unlodged crop varied from 2.8% and for

lodged crop were 1.6%. The mean cleaning efficiency for lodged and unlodged maize crop were 88.87 and 90.58% respectively. The grain damage for lodged and unlodged crop were 8.31% and 5.94% respectively. The Trash content for lodged and unlodged crop were 2.75 and 3.45% respectively. The maize combine performance was satisfactory with cutter bar header for maize crop at 1st low gear, forward speed of 2.10 Km.h^{-1} and reel rpm of 35. The maize crop residue after harvesting with cutter bar type maize header can be easily chopped and incorporated with disc harrow, rotary tiller etc. The performance of snap roll and cutter bar header with maize combine was also done. Total losses with snap roll header were higher as 15.06% and lower for cutter bar as 10.85%. The brokens were higher for cutter bar as 5.94 and lower for snap roll as 3.45%. The trash content was 3.45% for cutter bar header and 2.24% for snap roll header (Figure 14).

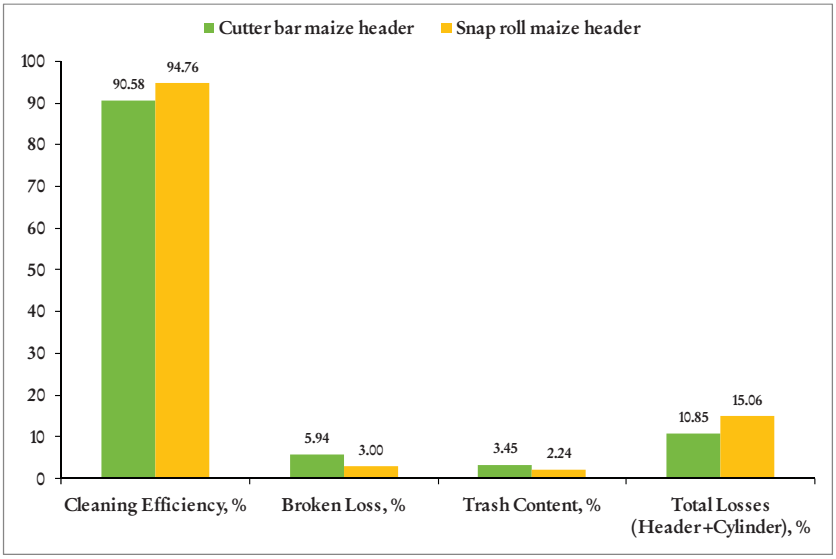

Figure 14. Graphical representation of field losses with combine harvester with cutter bar maize header and snap roll maize header

Undoubtedly header was more effective during turning at headlands as compared to snap roll type header owing to its independence from plant row spacings lacking of which in case of snap roll header causes a lot of gathering losses during turnings at headlands. Similar results were reported by Paulsen et al. (2014) in lodged maize crop. In the present study, 30-40% maize crop was lodged. Though the cutter bar type maize header was adjusted to nearly horizontal position yet the lodged crop was not picked completely. Cutter bar header passed over fully lodged crop without picking the cobs which lead to higher gathering losses for this header. Cutter bar header managed to pick cobs from those plants which though lodged but having cobs positioned at some height from ground. The lodged crop affects badly the working of any header mechanism during combine harvesting. The operator driving skill, header adjustment during field operation, combine forward speed with respect to reel speed, optimum maize crop moisture content (not too wet nor to dry) are the key factors which are needed to be given due importance before starting harvesting with combine so as to minimize various losses during field operation and better combine harvester performance. Particularly in case of lodged crop the field layout (from where to start) also plays an important role so that driver has an overview in mind how to operate effectively and adjust combine, reel and thresher speed during various sections of field so as to minimize field losses and maximizing the clean grain output. The developed cutter bar header cuts the maize plant from a certain height (adjustable) with minimum losses and feeds plant along with cob to the threshing unit of the combine. The maize header was capable of cutting the maize crop, irrespective of the width of the row and has higher field capacity as compared to snap roll header.

Thus a low cost effective cutter bar maize header was developed which is in the range of small and marginal farmers also and can be operated on custom hiring basis also. Moreover this header owing to its low weight can be operated with low HP combines with low repair and maintenance cost.

5. Conclusions

The performance of snap roll and cutter bar header with maize combine was also done. Total losses with snap roll header were higher as 15.06% and lower for cutter bar as 10.85%. The brokens were higher for cutter bar as 5.94 and lower for snap roll as 3.45%. The trash content was 3.45% for cutter bar header and 2.24% for snap roll header. This new type of developed cutter bar header can be used for harvesting maize crop efficiently and with minimum of losses as compared to snap roll header and maize dehusker cum sheller. Undoubtedly, the header was more effective during turning at headlands as compared to snap roll type header. Since, the header is independent of the width of the row, the gathering losses at the turning are much lower than those acquired in case of snap roll header. Though the cutter bar type maize header was adjusted to nearly horizontal position yet the lodged crop was not picked completely. Cutter bar header passed over fully lodged crop without picking the cobs which lead to higher gathering losses. Cutter bar header managed to pick cobs from those plants which though lodged but had cobs positioned at some height from ground. The operator driving skill, header adjustment during field operation, combine forward speed with respect to reel speed, optimum maize crop moisture content (not too wet nor to dry) are the key factors which are needed to be given due importance during combine harvesting. For minimizing various losses during field operation and better performance, particularly in case of lodged crop, the field layout (from where to start) also plays an important role. Therefore, the operator must have an overview in mind about how to effectively operate and adjust combine, reel and thresher speed during various sections of field thereby ensuring minimum field losses and maximum output.

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Technical and Field Evaluation of Tractor Operated Frontal Pre-pruner for Kinnow Mandarin (*Citrus reticulata*) and Guava (*Myrtaceae*) Orchard

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Abstract

Fruit tree pruning is the cutting and removing of selected parts of a fruit tree. It spans through quite a number of horticultural techniques. Pruning includes cutting branches back, sometimes removing smaller limbs entirely and more so the removal of young shoots, buds and leaves. Established orchard practice of both organic and nonorganic types typically includes pruning. Pruning can control growth, remove dead or diseased wood, and stimulate the formation of flowers and fruit buds. Pruning and training young trees improves their later productivity and longevity and can also prevent later injury from weak crotches or forks (where a tree trunk splits into two or more branches) that break from the weight of fruit, snow, or ice on the branches. However, the efficiency of pruning methods is also important. Manual pruning has constraints like lower field Capacity and incomplete pruning in case of tall trees. Therefore, a tractor operated 1-row frontal pre-pruner with electro hydraulic control was tested for Kinnow Mandarin and Guava orchards. The time involved for top and side pruning was 23.30 and 46.80 min/acre, respectively and there was 99.32-99.38% saving in time as compared to manual pruning.

Keywords: hydraulic, vertical, disc, horizontal, automatic, 1-row, Kinnow Mandarin, Guava, pre-pruner, top pruning, side pruning

1. Introcution

Mandarin orange (*Citrus reticulata*) is the most common citrus fruits grown in India. It occupies nearly 40% of the total area under citrus cultivation in India. The most important commercial citrus species in India are the mandarin (*Citrus reticulata*), sweet orange (*Citrus sinensis*) and lime lemon (*Citrus limon*) sharing 41, 23 and 23%, respectively of all citrus fruits produced in the country with an area of 0.428, 0.185, 0.286 million ha and production rate of 5.1, 3.27 and 3.15 million metric tons, respectively (Anonymous, 2018). Citrus (*Citrus* sp., Rutaceae) is cultivated in the states of Maharashtra, Andhra Pradesh, Punjab, Himachal Pradesh, Uttar Pradesh, Madhya Pradesh, Karnataka, Jammu and Kashmir, Orissa, Gujarat, Assam, Meghalaya, Rajasthan, Sikkim and Tamil Nadu. It is an evergreen medium to tall erected tree. It grows to a maximum height of 25 m (Anonymous, 2020a). In India, Guava (*Psidium guajava*, Myrtaceae) is successfully grown in Uttar Pradesh, Bihar, Madhya Pradesh, Maharashtra, West Bengal, Orissa and Tripura. Uttar Pradesh is considered as the most important guava producing state in India, and the Allahabad-Varanasi region has the reputation of growing the best quality guava in the country as well as in the world. It is a hardy and tall tree with height of more than 2.5 m (Anonymous, 2020b). In most developing countries, manual pruning are used whereas in developed countries mechanical pruner are used. Pruning is at the heart of arboriculture, one of the most important services arborists provide (Clark & Matheny, 2010). In the next future it is expected that greater use of wireless and lightweight equipment will be done to assess worker exposure to musculoskeletal disorders not only in pruning but in all farming operations (Elio et al., 2014). The results suggested that maintaining a live crown ratio of 55% would minimize effects of pruning on diameter growth. The effect of severe pruning on diameter increment was greater for subdominant trees than for dominant stems (Neilsen & Pinkard, 2011). In economic terms, if the rows are 400 m long, then the surface area suitable for mechanical pruning is 10 to 11 ha for all three varieties. There was no visible damage to the fruit branches with the mechanical pruner, but some damage occurred to wires with a diameter of less than 1.8 mm (Gambella & Sartori, 2014). Canopy management system labour operation cost

estimates indicated a labour saving of 62% and 80% with mechanical prepruning with hand shoot thinning (MPDHT) and mechanical box-pruning with mechanical shoot thinning (MPDMT) treatments, respectively when compared with hand pruning (HP) for 'Cabernet sauvignon' grape (Vitis vinifera). All treatments had similar yield, total soluble solids (TSS), juice pH, and titratable acidity (TA), Berry skin total phenolics, anthocyanins, and tannins at harvest. All treatments tested were within acceptable Ravaz index limits of 5 to 10 lb/lb. However, only MPDMT treatment reached a near optimum leaf area to fruit ratio of 1.2 m^2 kg^{-1} and pruning weight of 1.0 kg m^{-1} for warm climate viticulture (Kurtural et al., 2012). Sanding and pruning are two practices used in the cranberry (*Vaccinium macrocarpon*) industry for vine management and yield stimulation. Cumulative yield and net returns were higher in light severity treatments compared to those in the moderate and heavy treatments. Moderate and heavy sanding treatments were associated with lower yields and net returns than those for the untreated controls (Suhayda et al., 2009). Chancellor cited in Persson (1987), Kempe (1967) and Johnston (1968a and 1968b) also reported that the cutting force required when pruning a range of herbaceous (*Phleum pratense*) and woody (*Picea glauca*, *Pinus resinosa*, *Pinus banksiana*, *Pinus taeda*, *Pinus radiata*, *Abies balsamea*) up to 18 cm thick materials was greatly affected by the knife thickness. In some cases, doubling the knife thickness resulted in 50% increase in the cutting force. Mattson and Sturos (1996) on the other hand, found that knife thickness did not affect cutting force required to shear sugar maple (*Acer sacharum*) branches. This may have been due to lower cutting speeds (> 600 mm/sec compared with < 10 mm/sec) in the earlier study. Little agreement exists about the effect of cutting edge angle (*A*) on force and energy requirements. Kempe (1967) reported that a 45^o cutting edge angle require 20 to 30% less force to shear spruce logs than what was needed with a 60^o angle. Mattson and Sturos (1996) found that reducing the cutting edge angle (*A*) from 45^o to 30º resulted in a 55% reduction in peak force necessary for shearing sugar maple branches. Koch (1971) reported that a 9.5 mm thick blade with a 22.5^o cutting edge required 45% less total energy and 25% less peak force for shearing 130 mm diameter Southern Pine logs than was required when using a 45^o cutting edge (*A*). The counteredge angle has also been shown to affect peak force requirements. Chancellor (1957) stated that a "fine" counteredge requires approximately 25% less force than a "blunt" counteredge. Kempe (1967) reported that knives with recessed sides required 20% less peak force for the same cutting edge angle and thickness than parallel-sided knives. Koch (1971) found that tapered knives with a thin root also required less force. Koch (1971) and Johnston (1968b) both commented that small reductions in the necessary force could be achieved if the friction co-efficient between the blade and the wood was lowered. Greasing the blade has been found to have no appreciable effect but teflon coating of the blade surface is effective. The manner in which the force is applied has also been shown to have an effect. Mattson and Sturos (1996) demonstrated that an oblique cutting angle (β) requires greater peak force. They also examined the effect of cutting speed (600 vs 1100 mm/sec) on required force but found no significant difference at these high speeds. In summary, there performance may possibly be improved by tuning such design features as knife thickness, cutting edge angle (*A*), knife shape, knife friction, oblique cutting angle (13) and counteredge angle. Total energy and peak force required for cutting radiate pine and Douglas-fir branches were measured. Branch sizes ranged from 9 to 65 mm. Under one set of standard conditions some shears required over 50% more energy and peak force than others. Douglas-fir required more energy and force than radiate pine. Total energy and force requirements tend to increase with cutting edge angle and with blade thickness (Crossland et al., 1997). The selection of pruner machine is also dependent on type of orchard. In general intensive/high density orchards (HD system) are characterized by densities between 250 and 700 trees per ha, super-high-density systems (SHD system) orchards can present densities over 1500 trees/ha (the hedgerow system). The average full yield in high density systems ranges between 6000-10000 kg/ha for rainfed and irrigated orchards. However the economic life of the SHD is shorter (around 15 years, while in intensive system it can be more than 30 years) due to the lack of space and the competition among trees for light and ventilation inside the canopies (Freixa et al., 2011). Intensive tree orchard with narrow tree canopy or even 2D planar fruiting wall would be suitable for fully autonomous pruning system in the future. With the adoption of intensive tree architecture as well as the improvement of cutting end-effector, tree branch identification and reconstruction, it is very promising to have a robotic pruning system for tree fruit crops (He & Schupp, 2018). A remote operated system may be an operating alternative for pruning equipment although there are remote control systems developed in the United States, Canada and Israel (Castellanos et al., 2017). A study was done to determine the input requirements for both the hydraulic circuit and the mechanical pruner designs. Then a description of an adapted inter-axle carrier used for the experimental model of the hop mechanical pruner and of the effected field measurement follows, along with interpretation of the measured data. These data are depicted in clearly arranged graphs showing the dependency of pressure and hydraulic oil flow on the cutting disc rotational frequency (Hoffmann et al., 2015).

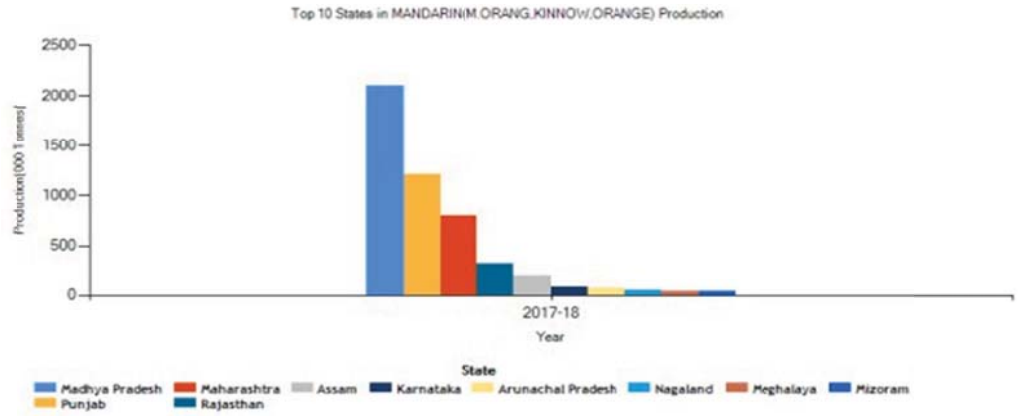

Figure 1. Major Mandarin producing states of India

Source: http://apeda.in/agriexchange/India%20Production/India_Productions.aspx?cat=fruit&hscode=1064

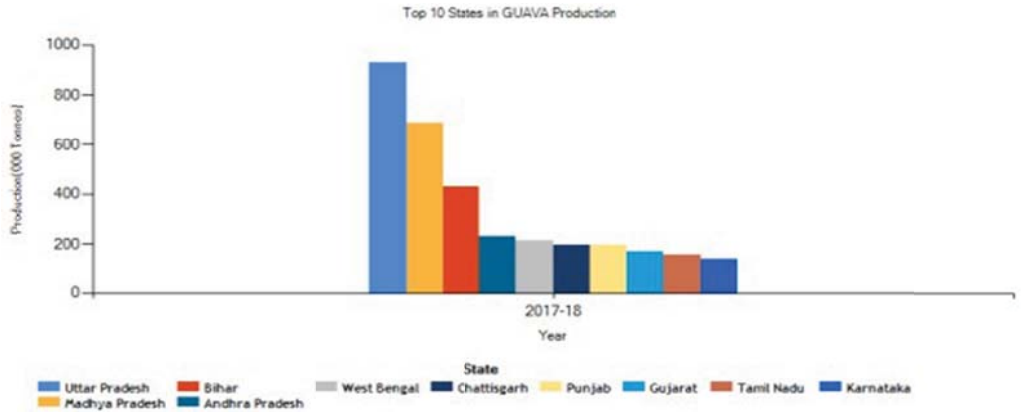

Figure 2. Major Guava producing states of India

Source: http://agriexchange.apeda.gov.in/India%20Production/India_Productions.aspx?cat=fruit&hscode=1046

Major Kinnow and Guava producing states in India are shown in Figures 1 and 2. The total area under Kinnow crop was around 53,045 ha which accounts for 60% of the total area under fruits in Punjab. The production of Kinnow was 1,246,821 MT. The area under Guava was 9142 ha with a production of 206106 MT (Thind & Mahal, 2019). Manual pruning has constraints like lower field capacity and incomplete pruning in case of tall trees. Therefore a mechanical pruner can overcome both of these constraints. Therefore, a pre-pruner was tested for Kinnow and Guava orchards in Indian conditions.

2. Material and Methods

2.1 Experimental Site Detail

In 2017, a pre-pruner machine was operated at Kinnow and Guava orchards at Punjab Agricultural University, Ludhiana. The field evaluation of tractor operated pruner was done for Kinnow and Guava orchard at a spacing of 5 m by 7.5 m (plant spacing × row spacing) and height of Kinnow Mandarin and Guava tree varied from 3.66 m to 4.27 m with an average plant population of 275 ha^{-1} (intensive/highdensity system). A view of Kinnow Mandarin orchard is shown in Figure 3.

Figure 3. A view of Kinnow Mandarin orchard

2.2 General Description of Pre-pruner

Pre-pruner (Figure 4) machine is equipped as standard with hydraulic motors facilitated with safety system against stresses, cutting module (discs) of low maintenance without pulleys or belts, mechanical regulation of the angle of incidence of the cut, saws disks (Figure 5), inclination and hydraulic positioning of the cutting plane, hydraulic lifting, hydraulic lateral displacement, hydraulic power station and chiller. Its design is especially indicated for work in orchard traditional crops where the space between plants is reduced. Saw blades are 600 mm in diameter, with the availability of special discs for very thin branches or pruning in green. It requires a minimum power of 40 hp tractor. The table is rotating to position the cutting module to the right or left of the tractor. It has a maximum cutting diameter up to 12 cm with a maximum cutting height in horizontal position of 4 m and minimum of 1.6 m. The maximum height of vertical cut up is 7.15 m and 3.7 m down (pendulum). The machine high performance is due to its ease of operation and cutting positions with more than 250° of travel in the position of the cutting module. On the PFS-5 (Reinforced orchard disc pruner compact) models the coupling to the tractor is front with number of hydraulic functions of 3 + 1 and 4 + 1. Discs RPM are 1650 rpm but it also depend on the branches to be cut and the discs mounted on the machine. Different kind of discs can be used to cut different diameter of branches.

Figure 4. A view of pre-pruner

Figure 5. A view of cutting discs

The pre-pruner is very high-tech fully hydraulic equipment and is equipped with individual hydraulic motors in each of the discs, therefore with total absence of pulleys or belts. Moreover its independent motor in each disc equipped with a safety valve and automatic reset in case of blocking, gives a great power of cut with a very reduced maintenance. It can be adapted to the front of the orchard's tractors allowing cutting both on the sides and at the top of the tree. Its design is especially indicated for work in orchard traditional crops. It is equipped with a turning frame which facilitates to work with the cutting bar positioned to the right or left of the tractor, being able to determine the cutting direction and the place of evacuation of the branches. Due to its high speed of turning, it gives a high cutting quality and correct evacuation of branches outside the tree. High quality of work and safety both in its handling as in the mechanical and hydraulic integrity of the same since it has individual systems of protection of the motors and rest of the hydraulic components.

2.2.1 Main Technical Characteristics of Standard Equipment

- ✓ Lift inclination and hydraulic extension.
- ✓ Electric controls in cabin.
- ✓ 1 Motor + safety valve on each disc.
- ✓ Hydraulic lifting.
- ✓ Hydraulic sideshift.
- ✓ Hydraulic inclination.
- ✓ Manual turning central frame (cutting left/right and branches discharge).
- ✓ Hardened steel 86 teeth-disc.
- ✓ 150L Hydraulic power unit with oil cooler and lateral counterweight box (C16).
- ✓ Power supply by independent hydraulicpower station to tdf.
- ✓ Front coupling to tractor.
- ✓ Reverse hydraulic device of the cutting module. Other cutting heights on demand.
- ✓ Additional modules for cutting skirts.
- ✓ Special module for pruning on the sides of the tree.
- ✓ Electronic speed control of the discs (Figure 5) in both directions.
- ✓ Integrated automatic control of cutting functions with programming in work position memories.
- ✓ Different types of cutting discs.
- ✓ Safety system with automatic reset.

Storage foot includes:

- ✓ Adaptable standard plate to tractor (frontal).
- ✓ Lateral support rods.

The tilt adjustment of the disc module is by electric control installed in a cab (Figures 6, 7 and 8), and has a mechanical adjustment of the angle of incidence. It is a very high-tech integral hydraulic drive machine. The standard dimensions of linkages of pre-pruner machine in various positions are shown in Figure 9.

Figure 6. A Schematic view of various components of pre-pruner

Figure 7. A view of tractor operated pre-pruner lab and field experiment

Figure 8. Pressure gauge for checking hydraulic oil pressure

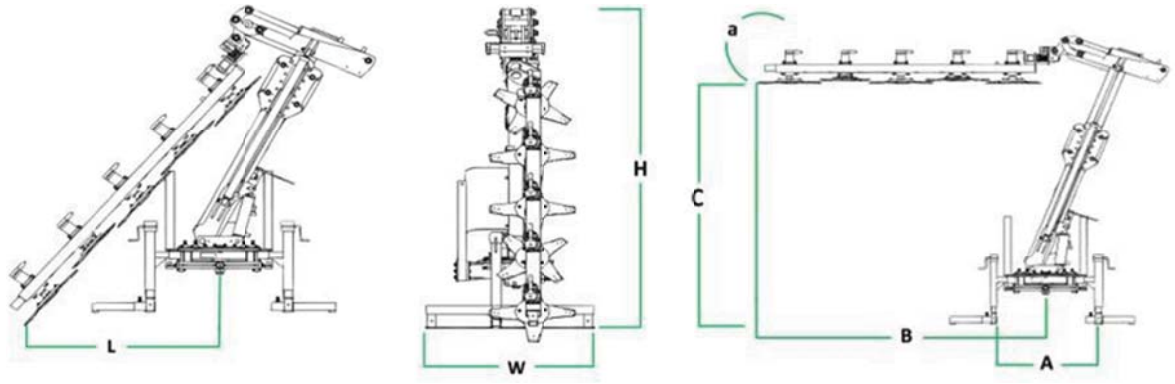

Figure 9. Various linkage dimensions of pre-pruner

Table 1. Various technical specifications of pre-pruner

Particulars	Symbols	Detail
Tractor HP required		≥ 44.76 kw
Battery power and current required to operate distributor (driving various hydraulic mototrs)		12 V, 6 A
Switches for horizontal positioning of pre-pruner		One
Switches for multiple positioning in horizontal mode		Three
Switches for vertical positioning of pre-pruner		One
Switches for multiple positioning in horizontal mode		Three
Model		PFS-V5XX-2750, compact XXL frontal pre-pruner
Type		Frontal pre-pruner
Movement		Cut, lifting movement, tilting module
Diameter discs Nº/mm		5, ϕ600 mm (40 toothed) thickness of disc-3 mm
Cutting disc tours/minute or revolutions per minute (rpm)		1650 t/m
Cutting length		2750 mm
Hydraulic functions		4
Movements Nº		3
Weight, kg		920
A, mm		770
B, mm		Min 2700, Max 4150
C, mm		Min 2700, Max 4200
H, mm		2900
W, mm		1170
L, mm		2600

To make the machine functional it was necessary to develop the control box indigenously. Material required to develop control box included switches, switch boards, ICB, hydraulic pipes, wires etc and this indigenous control box was designed and developed with the support of local industry. Electro hydraulic control has been made by electro valves connected through a control box.

2.3 Estimation of Field Capacity

The effective field capacity was determined by measuring all the time elements involved while harvesting. The total time was categorized into the productive and non-productive time. The productive time is the actual time used for harvesting the grains while the non-productive time consisted of the turning time, repair and adjustment time and other time losses. The area covered divided by the total time gave the effective field capacity. The effective field capacity of combine was calculated using the following formula (Kepner et al., 1978):

$$C = \frac{SW}{10} \times \frac{E_f}{100} \quad (1)$$

where, C: effective field capacity, ha h^{-1}; S: speed of travel, km h^{-1}; W: rated width of implement, m; E_f: Field efficiency, in percent.

$$E_f = 100 \times \frac{T_0}{T_e + T_h + T_a} \quad (2)$$

where, T_0: theoretical time per hectare (per acre); T_e: effective operating time = $T_0 \times 100/K$; K: percent of implement width actually utilized; T_h: time lost per acre due to interruptions that are not proportional to area. At least part of T_h usually tends to be proportional to T_e; T_a: time lost per acre due to interruptions that tend to be proportional to area.

2.4 Estimation of Fuel Consumption

Before starting the test, the engine's fuel tank was completely filled. The quantity of fuel required to fill the tank after harvesting the test field was measured using a 1 *l* graduated cylinder. Thus, the fuel consumed during the test was determined.

$$F = L/A \quad (3)$$

where, F is the fuel consumption in l ha^{-1}; A is the area harvested in ha; and L is the quantity of fuel required to fill the tank after harvesting the test field in *l*.

2.5 Economics

For economics calculations labour cost, diesel cost, repair cost etc. were considered. The economics was worked out for comparing the tractor operated pre-pruner savings as compared to manual pruning operation.

2.6 Statistical Analysis

The Two way Anova was used for statistical analysis of data. The two-factor Anova with replication was applied on field data.

3. Results and Discussions

Field evaluation of pre-pruning machine was done for Kinnow at new orchard, PAU, Ludhiana, 2017. Other orchard specifications and operational parameters are shown in Table 2.

Figure 10. Sequential view of tractor operated pre-pruner in vertical cutting position and view of cut branches

Table 2. Orchard specifications and operational parameters of pruner for Kinnow Mandarin and Guava orchard

Particulars	Range/Mean
Average forward speed of Pruner, Km h^{-1}	2.73
Fuel Consumption, l h^{-1}	5.0-6.0
Canopy width between pruning, m	2.52
Canopy width after pruning, m	1.94
Canopy height before pruning, m	4.11
Canopy height after pruning, m	3.00
Uncut lower branch height, cm	63-78
Cut branch diameter/thickness, mm	5-25

The field layout was prepared prior to each operation in orchard for maximizing field efficiency and minimizing time lost in turnings. The time of travel for each straight row and time involved in turnings were recorded for each orchard field capacity calculations. The speed of cutting discs and inclination of cutting bar was controlled by electronic panel, distributor and hydraulic mototrs provided for each saw blade.

The machine was operated on side and top and side of Kinnow Mandarin and Gauava plants (Figures 10 and 11) and time of operation for both kinds was recorded and is shown in Table 3.

Figure 11. Top Pruning in Guava and Kinnow Mandarin orchard and view of cut branches in Guava orchards

The time involved in pruning for kinnow orchard was 40.86 and 23.30 min/acre for side and top pruning. The pruning time involved per tree for side and top pruning was 19.46 seconds and 11.10 seconds respectively. The number of plants in one row was 21.

Table 3. Mean time for side and top pruning of Kinnow Mandarin and Guava orchards

Method of pruning	Mean time for pruning/tree		Mean time for pruning/acre		Saving in time
	Side	Top	Side	Top	
Tree Pruner	19.46 Seconds	11.10 seconds	40.86 min.	23.30 min.	99.32-99.38
Manual Pruning	60-90 min.	Not possible	126-189 h	Not possible	-

Table 4. Effect of pruning method on time of side pruning for a single tree and for an acre field (including turning time)

Parameter	Method	R_1	R_2`	R_3	R_4	R_5	Mean
Time for pruning per tree, seconds	Pre-pruner	15.29	22.86	22.71	20.43	16.00	19.46 seconds
	Manual pruning	171.43	228.57	214.29	234.29	257.14	221.14 seconds
Time for pruning per acre, min/acre	Pre-pruner	32.10	48.00	47.70	42.90	33.60	40.86 minutes
	Manual pruning	7560.00	8400.00	9600.00	10800.00	11340.00	9540.00 minutes

Table 5. Results of two factors Anova with replications

Source of variation	SS	df	MS	F	P-value	F crit
Sample	117632531.4	1	117632531.4	187.1728	3.01E-10	4.493998
Columns	109050524.4	1	109050524.4	173.5174	5.27E-10	4.493998
Interaction	108053313.6	1	108053313.6	171.9306	5.64E-10	4.493998
Within	10055525.93	16	628470.3706			
Total	344791895.3	19				

Table 6. Time involved in top pruning with pre-pruner for single tree and for an acre field (including turning time)

Parameter	Method	R_1	R_2	R_3	R_4	R_5	Mean time per row	Mean time per tree	Mean time per acre
Time for pruning per tree, seconds	Pre-pruner	236	171	295	267	196	233 seconds	11.10 seconds	-
	Manual pruning	-	-	-	-	-	-	-	-
Time for pruning per acre, min/acre	Pre-pruner	23.60	17.10	29.50	26.70	19.60	-	-	23.30
	Manual pruning	-	-	-	-	-	-	-	-

The saving of time for pre-pruner as compared to manual pruning varied between 99.32-99.38%. For side pruning two sides were considered and for top pruning only one side was considered. The time for turning of pre-pruner was measured as 60 seconds and was added in both times involved in side pruning and top pruning. The effect of pre-pruner and manual pruning on pruning time per tree and pruning time per acre are shown in Table 4 and results of statistical analysis are shown in Table 5. The data for time involved in top pruning of tree is shown in Figure 6. The effect of pruning method was significant on time involved in side pruning per tree and per acre ($p < 0.05$). Some authors, such as Kallsen (2005), compared several types and intensities of mechanical pruning, such as topping at several heights and some hand-pruning intensities, with non-pruning. He noticed that, in all cases, the higher the pruning intensity was, the lower the yield was, regardless of the type of pruning used. In the same way, Joubert et al. (2000), working in South Africa, tested the effect of light and severe prepruning followed up by hand pruning in 'Valencia'and 'Navel' oranges and 'Star Ruby' grapefruits. After three years'experimentation, they were able to confirm that all the systems tested produced a higher yield than the unpruned control, with the best choice being hedging with an inclination of 10-20° combined with hand pruning once or twice a year. Pre-pruning in which a tilted plane is produced facilitates lighting of the bottom of the tree and also favours the concentration of fruits in the lower part of the tree, which makes manual harvesting easier. Spanish citrus farmers like to leave the trees with large skirts because this is a highly productive part of the tree. However, skirting tests performed with prepruners have shown that the overall production of the tree is not affected, while mechanical harvesting is facilitated, problems with soil fungus are reduced and tree microclimate is affected (El-Zeftawi, 1976; Morales et al., 2000; Sauls, 2008). Nowadays, mechanical pruning, either alone or combined with hand pruning, is used by some Spanish farmers. It is, however, not a technique that is widely accepted by growers, among other reasons due to a lack of experience. Pruning citrus trees must be a general canopy management strategy based on the understanding of specific pruning and regrowth management practices that must be combined with cost-effective methods adapted to each orchard period, growth, full production and old trees decline due to age and/or shading (A. J. Krajewski & S. A. Krajewski, 2011). So economics part was also calculated for the pre-pruner operation keeping in view its future scope.

4. Economics

The economics calculation was also done for pre-pruner and manual pruning method. The operational cost for pre-pruner and manual pre-pruner were calculated taking into account their field capacities. The field capacity was calculated using all the time involved in pruning

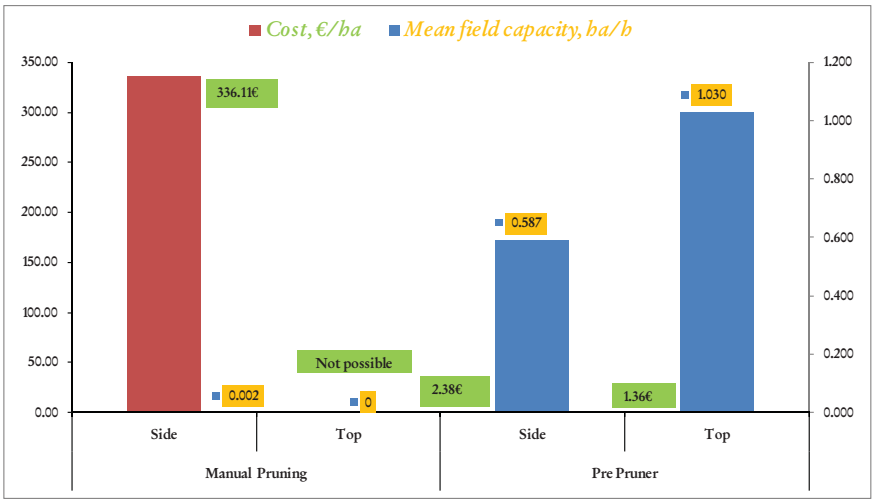

Figure 12. A graphical representation of field capacity and cost of pruning operation for pre-pruner and manual pruning operation for manual method

For pre-pruner machine time lost in turnings, breakdowns etc were taken into account. The labour cost (0.61 €/h), diesel cost (0.79 €/L), repair costs were considered for calculations. The cost of operation for side pruning operation for pre-pruner and manual pruning were calculated as 2.38 €/ha and 336.11 €/ha respectively (Figure 12; 1 € = 82.19 INR). The cost of top pruning for manual method was not worked out as it was not possible for this method. The cost of top pruning in case of pre-pruner was worked out as 1.36 €/ha. The saving in cost with pre-pruner machine for side pruning was 99.29% with added advantage of complete top pruning of tall fruit plants/orchards.

5. Conclusions

The mean field capacity of manual pruning was calculated as 0.002 ha h^{-1} for side pruning and for top pruning the manual efficiency was very low and unable to complete the pruning due to reach problem. Whereas in case of tractor operated pre-pruner the mean field capacity was higher for side and top pruning as 0.587 ha h^{-1} and 1.030 ha h^{-1} respectively along with benefits of efficient and complete pruning. Orchard planting geometry should be such as to facilitate easy, quick and balanced movement of tractor in between rows and at headlands, *i.e.*, orchards should have minimum of ridges, undulation as well as weeds/grasses to avoid slippage of tractor during operation and for maximizing field capacity of machine. The irrigation planning of orchard should be done keeping in view the date of next pruning operation or should be well in advance before pruning operation so that during pruning field is in dry conditions this so to provide good traction condition for tractor during operation. The operator should be fully aware about controls of pre pruning machine and equipped with good driving skills which are very important for field operation and road movement of pre pruning machine.

Tractor with cabin should be preferred for pre pruning machine for safety of operator as during pruning of trees small wooden pieces may hit and cause injury to the tractor operator. For plants having height more than 10 feet high clearance tractor may be used for enhancing visibility of operator during field operation. The pruning of tree sides should be done before start of top pruning. The choking may occur in pre pruning machine for the case when machine is lowered to increase cutting height of top portion greater than overall height of cutting unit. Therefore cutting height for top portion should be selected accordingly.

6. Before Starting Operation Operator Should Check These Points Daily

✓ The oil level in the reservoir should be checked for marked level and if found less should be filled up to marked level first.

✓ Nuts of all the rotating blades and other units should be checked and tightened if needed.

✓ The free movement of pre pruning machine should be checked in all planes.

✓ The rotary blades should be checked by running them ideally.

✓ All the hose pipes should be checked for any leakage and if found should be repaired or replaced before operation.

✓ The support system of should be checked thoroughly for any loose nuts and if found any should be tightened.

✓ The wearing of cutting discs also depend on the branches diameter but normally they need sharpening before starting a new season.

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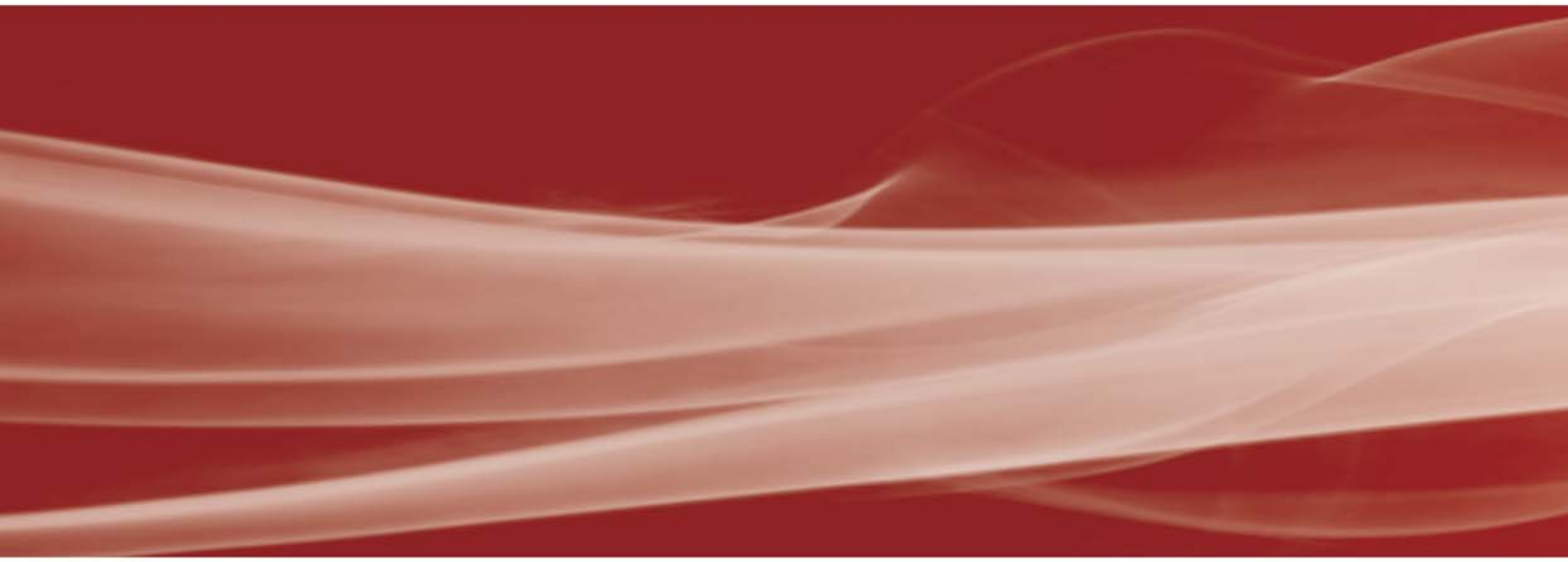

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