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Full Length Research Paper

Morphological characteristics of mycelia growth of two strains of the indigenous medicinal mushroom, *Lentinus squarrosulus* Mont. (Singer), on solid media

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Morphological characteristics of the mycelia growth of two wild strains of *Lentinus squarrosulus* Mont. (Singer), *Sqw* and *Lsf*, collected from the Volta and Greater Accra regions of Ghana respectively, were studied. Growth characteristics including mycelia growth rate and mycelia density and morphology by the 7th and 26th days of incubation on solid media formulated from four lignocellulosic wastes: elephant grass (EG), rice straw (RS), thatch (TH) and sawdust were classified. Tissue cultures of the fruit bodies were prepared on Malt Extract Agar (MEA). Eight-day old cultures of the subsequently prepared 1st generation cultures were inoculated on the media and incubated at 25°C. Strain *Sqw* recorded lower growth rates (between 0.47 and 0.64 cm/day) on the solid media than *Lsf* (between 0.69 and 0.93 cm/day). The maximum growth rate of strain *Sqw* was 0.64 cm/day on both SD and TH whereas that of *Lsf* was 0.93 cm/day on EG. *L. squarrosulus* mycelia density is not dependent on the growth rate and vise-versa, irrespective of the strain. Although both strains generally had the longitudinally radial morphology with concentric rings, with extended incubation, the culture morphology of both strains changed, usually into thick mats. Mycelia of both strains on all the media were white at the initial stages of incubation. Cultures of strain *Sqw* largely remained whitish and turned brownish only on EG, whereas strain *Lsf* turned into different shades of brown on all media with extended incubation. These colour changes were not uniform on the entire plate, appearing in undefined sectors. Mycelia growth characteristics were seen to be substrate and strain-dependent. Futher investigations of these observations could uncover some behavior of *L. squarrosulus* such as changes in enzyme profile and the phenolic content, which could have applications in biotechnology.

Key words: Culture morphology, growth rate, mycelia density, indigenous, lignocellulosic waste, solid media, strains.

INTRODUCTION

The human diet is very diverse, and includes products ranging from plants to seafood to other animals. Mushrooms are fungi, which have occupied a part of this diversity in the human diet over centuries, due to their nutritional, medicinal, and organoleptic properties including aroma, flavor and taste (Chang, 1998; Zawirska-Wojtasiak, 2004). Fruit bodies of mushrooms were mainly collected from the wild in their natural habitats in the past.

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However, due to increasing demand and problems such as hallucinations, allergic reactions and sometimes death caused by ingestion of inedible and/or poisonous mushrooms collected by inexperienced collectors, the need arose to develop technologies to ensure the production of edible and/or medicinal mushrooms. So far, *Agaricus* spp. are the most cultivated mushrooms worldwide (Chang, 1998). Other cultivated mushrooms include members of the genus Auricularia (Tang et al., 2010), Pleurotus (Cohen et al., 2002; Obodai et al., 2011; Royse, 1992) and, to a lesser extent, Lentinus (Adesina et al., 2011; Mhd Omar et al., 2011; Nwanze et al., 2006; Pukahuta et al., 2008).

Lentinus squarrosulus Mont. (Singer) is a white rot fungus of the order, Polyporales. The mushroom is usually found in the wild on dead or decaying wood (Karunarathna et al., 2011) of various trees. It is widely known as an indigenous mushroom in Nigeria (Adesina et al., 2011; Nwanze et al., 2005), having been reported to have various organoleptic, nutritional and medicinal attributes (Mhd Omar et al., 2011; Nwanze et al., 2006). *L. squarrosulus* has been reported to be rich in proteins, sugars, lipids, amino acids, vitamins B, C, and D, and minerals (Mhd Omar et al., 2011; Royse et al., 1990). According to Mhd Omar et al. (2011), liquid mycelia extract of the mushroom have ulcer prevention and healing capabilities in rats.

Despite the diverse research output on *L. squarrosulus* cultivation on various substrates under various conditions (Adesina et al., 2011; Mhd Omar et al., 2011; Nwanze et al., 2005; Okhuoya et al., 2005), nutritional composition and medicinal properties (Bhunia et al., 2011; Bhunia et al., 2010; Mhd Omar et al., 2011), toxicological evaluation (Kadiri, 2005), enzyme profile (Mtui, 2012; Tripathi et al., 2012; Wuyep et al., 2003), and effect of different media on the mycelial growth of submerged cultures (Subramonian et al., 2010), there is very little information on the mycelia morphological characterization on solid media. However, Atri and Lata (2013) have reported on the vegetative growth of *Lentinus cladopus* Lév on both commercial solid and liquid media including Malt Extract Agar, Potato Dextrose Agar, Pea Extract Agar, Potato Malt Agar, Malt Broth, Potato Dextrose Broth, Czapek Solution, Glucose Asparagine Medium, Glucose Peptone Medium, and Dimmick Medium.

According to Chang (1998) the important steps preceding the development of active spawn for mushroom cultivation include selection of an acceptable mushroom species and secreting a good quality fruiting culture. In addition, because of the known phenomenon of species-specificity of fruiting mycelium, the minimal information on the mycelia morphology of *L. squarrosulus* on solid media is considered a problem even though Stamets and Chilton (1983) have indicated that rhizomorphic and longitudinally radial mycelia are more likely to produce primordia than cottony mycelia. This is more so because the culture behavior of mushrooms has been reported (Sharma and Atri, 2013) to be directly linked with cultivation and pharmaceutical aspects of the mushrooms.

This report therefore seeks to characterize and document the mycelial behavior of two strains of indigenous *L. squarrosulus* on various solid media formulated from four lignocellulosic wastes: elephant grass (EG), rice straw (RS), thatch (TH), and sawdust, in terms of their mycelia growth rates, densities, and morphologies.

MATERIALS AND METHODS

Mushroom strain

L. squarrosulus strains from two sources were used; *L. squarrosulus* strain *Sqw* (Figure 1A) collected on a mango tree during a field trip at Wli Agumatsa waterfalls, a suburb at the Volta Region of Ghana, and strain *Lsf* (Figure 1B) collected on the log of a jack tree at the CSIR-Food Research Institute premises in Accra. Identification of the mushrooms was done with macro-morphological features. Samples of fresh fruit bodies of both strains were used in the preparation of tissue cultures.

Mushroom tissue culture preparation

Malt Extract Agar (MEA; OXOID^{TM} Ltd., England) was prepared and sterilized according to the manufacturer's instructions but with supplementation of the carbon source with dextrose (20 g/L). Each poured plate of MEA was aseptically inoculated with an aliquot of the inside tissue from the region between the cap and stipe of fresh *L. squarrosulus* fruit bodies. Plates were incubated in the dark at 25°C in an incubator (Tuttlingten™ WTC Binder, Germany) to obtain the tissue cultures. Upon complete colonization of the MEA (8 days of incubation), the tissue cultures were sub-cultured on MEA plates and incubated under the same conditions as the tissue cultures. This culture, referred to as the 1st generation culture, was used for further studies.

Solid media preparation and inoculation

Solid media were prepared from dried and chopped straw-based lignocellulosic wastes (elephant grass (EG), rice straw (RS) and thatch (TH)), and fresh sawdust respectively. Substrates (75 g each) were separately soaked in 1.1 L of tap water for 1 hour (to allow leaching of nutrients from the substrate into the water), after which the supernatants were decanted and filtered through cotton wool into a measuring cylinder. To each of the filtrates, 17.5 g agar and 20 g glucose were added and stirred. The resulting solutions were separately brought to a boil on a hot plate while stirring (to avoid lump formation). The prepared solid media were distributed into clean conical flasks after ensuring the agar and glucose were well dissolved in the solution (about 5 mins of boiling). All solid media were sterilized at 121°C for 1h. Cooled solid media were aseptically poured into the required number of 9 cm diameter petri dishes and allowed to set. The various substrates were each inoculated with 1 cm^2 disks of 8-day old 1st generation cultures. The experiment was performed in triplicates.

Measurement of mycelia growth rates and mycelia densities

Radial mycelia growth was measured by taking daily readings of mycelia extensions from the center of the inocula on the plates in two perpendicular directions with a ruler. This was taken initially on

Figure 1A. Pictures of *l. squarrosulus* strain (A) *Sqw*.

Figure 1B. *Lsf* in their natural habitat.

the third day of incubation and consequently after every 24 h for 4 days. Readings were taken in the same perpendicular angle each time and the readings were taken following the same sequence each day. Recorded values per day are means of the two readings taken at the perpendicular angle.

Mycelia densities were recorded by direct observation of the plates based on the thickness of the mycelia as judged by the observers. The mycelia densities on the plates were rated from 1 to 5 with 1 being the least dense and 5 being highly dense. Least dense mycelia were defined as mycelia, which are barely visible on the solid media and are transparent such that the media can be observed through the mycelia when observed from the cover of the plate. On the other hand, highly dense mycelia were defined as mycelia, which are very thick and are opaque such that the solid media cannot be seen through them when the plate is observed from the cover. In case of plates with sectored mycelia, the mycelia density was judged based on the density of the most prominent (with regards to size of sector) mycelia on the plate.

Statistical analysis

Data obtained from triplicates were analyzed with GenStat Discovery Software (4th Edition). The table of correlations was generated with the software. Mycelia growth rates were obtained from linear trendline equations from the plot of the incubation period versus the radial mycelia extension averaged from the triplicates of each setup.

RESULTS AND DISCUSSION

Mycelia growth characteristics

Wide variations of the radial mycelia extension of *L. squarrosulus* strain *Sqw* on the various substrates studied on the 3rd day of incubation were obtained among the triplicates for all the substrates (Figure 2A)**Error! Reference source not found.**. The mean values recorded were negatively skewed on all the substrates for the strain and the mycelia extension on EG was significantly lower than that observed on RS and TH by the third day of incubation. Mycelia growth on SD did not differ significantly from that recorded on the other substrates (EG, RS and TH).

The radial mycelia extension of *L. squarrosulus* strain *Lsf* on the various substrates on the third day of incubation from the triplicates on EG and TH had minimal variation (Figure 2B). Mycelia growth of the strain was significantly fastest on EG followed by the growth on RS, whereas slower growth was observed on SD and TH (Figure 2B). *L. squarrosulus* strain *Lsf* was more vigorous under the study conditions, showing longer mycelia extensions than *L. squarrosulus* strain *Sqw* on the solid media by the third day of incubation (Figure 2A and B). No outliers were recorded among the data sets obtained for both strains (Figure 2A and B; outliers would be indicated by green or red asterisks on the affected box and whisker in the graphs).

The mycelia growth rates of *L. squarrosulus* strain *Sqw* (Figure 3A) on the solid media were 0.64, 0.64, 0.60 and 0.47 cm/day on SD, TH, RS and EG respectively. However, mycelia growth rates of *L. squarrosulus* strain *Lsf* on the substrates (Figure 3B) followed a different pattern and the growth rates on EG, RS, TH, and SD were 0.93, 0.91, 0.78, and 0.69 cm/day respectively.

The observed trend of different mycelia growth on various solid media has also been reported in another study on *L. cladopus* Lév (Atri and Guleria, 2013). According to the authors, taxonomically *L. cladopus* is quite close to *L. squarrosulus* Mont. in having a similar morphology and hyphal construction but differs from it in having thin pileus, lacking squamules and presence of broader spores. However, the recorded growth rates of both strains on all the solid media studied were lower than that recorded (1.8 cm/day) for indigenous Indian *L. squarrosulus* grown on Potatoes Dextrose Agar (PDA) at 27±1°C (Sharma and Atri, 2013). The discrepancy observed could be attributed to the growth media used, the incubation temperature or a combination of both factors.

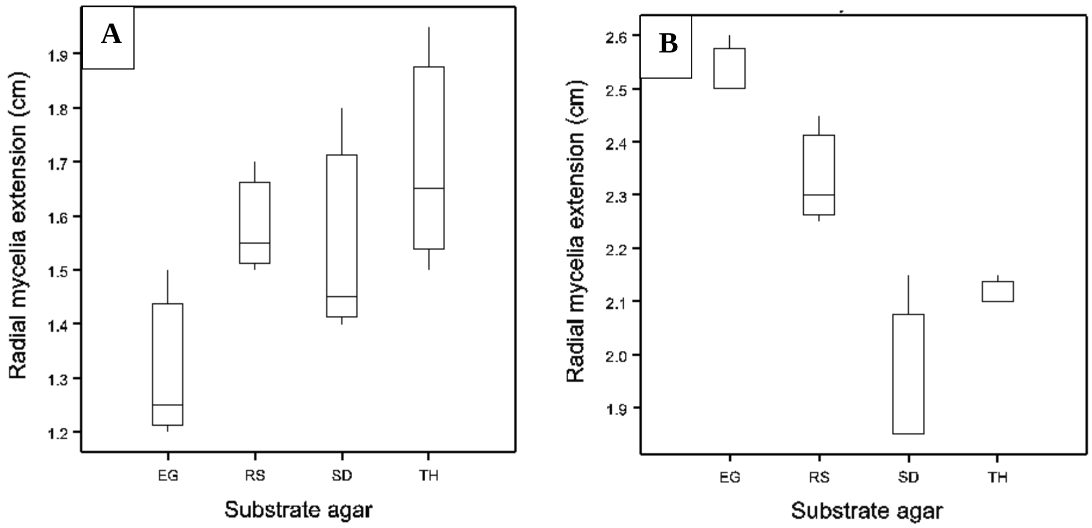

Figure 2. (a) Radial mycelia extension of *L. squarrosulus* strain Sqw on third day of incubation in relation to solid media. n=3. (b) Radial mycelia extension of *L. squarrosulus* strain Lsf on third day of incubation in relation to solid media. n=3.

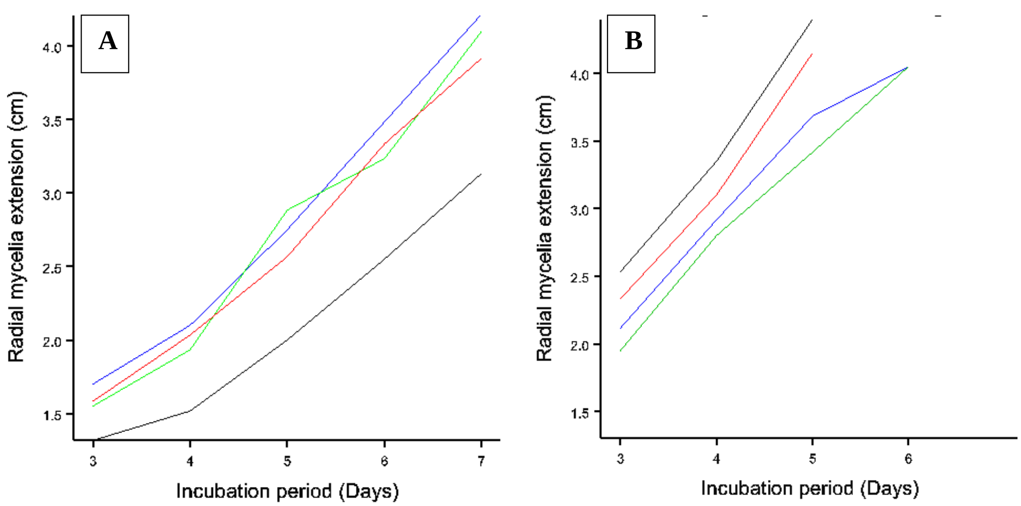

Figure 3. (a) Mycelia growth of *L. squarrosulus* strain Sqw within one week of incubation. n=3. and (b) Mycelia growth of *L. squarrosulus strain* Lsf within one week of incubation. n=3.

The results of this study demonstrate that *L. squarrosulus* media preference is strain specific.

Mycelia morphologies on solid media

The mycelia morphologies of the strains, presented in Figure 4 and Figure 5, varied on the various solid media under the conditions employed in this study.

Culture morphology of *L. squarrosulus* strain *Sqw*

On EG, the mycelia showed linear growth with clear concentric rings and relatively dense mycelia (Figure 4A). While the linear growth with concentric morphology was also observed on RS and TH (Figure 4B and C respectively), the rings were not as pronounce as that observed on EG (Figure 4A). The prominence of the concentric rings on these solid media was in the order, EG>RS>TH. The culture morphology on RS was relatively uniform in comparison to that observed on TH (Figure 4B and C respectively). There were no concentric rings observed on SD (Figure 4D). On this solid media, a linear morphology with low mycelia density was observed

(Figure 4D). The mycelia were white on all solid media from the beginning of incubation through to the 7th day of incubation (Figure 4A, B, C and D) and throughout the period of substrate colonization (all solid media were fully colonized by the 10th day of incubation; data not shown).

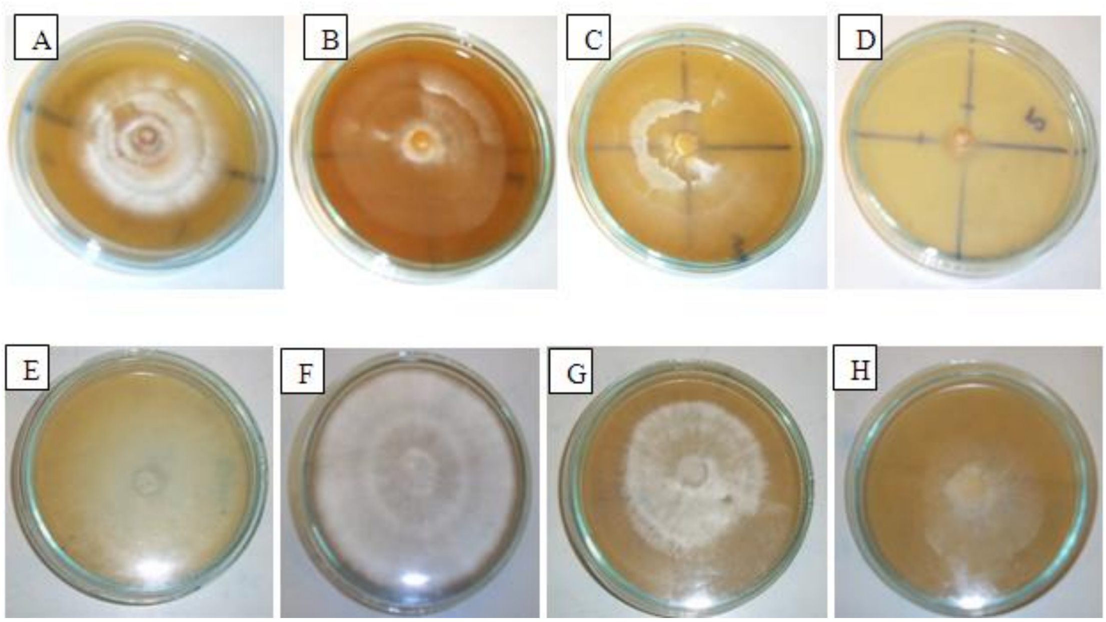

Figure 4. *L. squarosulus* strains *Sqw* and *Lsf* (top and bottom rows respectively) colony morphology on the 7th day of incubation (young cultures) at 25°C on various solid media. A and E, B and F, C and G, and D and H represent the strains inoculated on EG, RS, TH, and SD respectively.

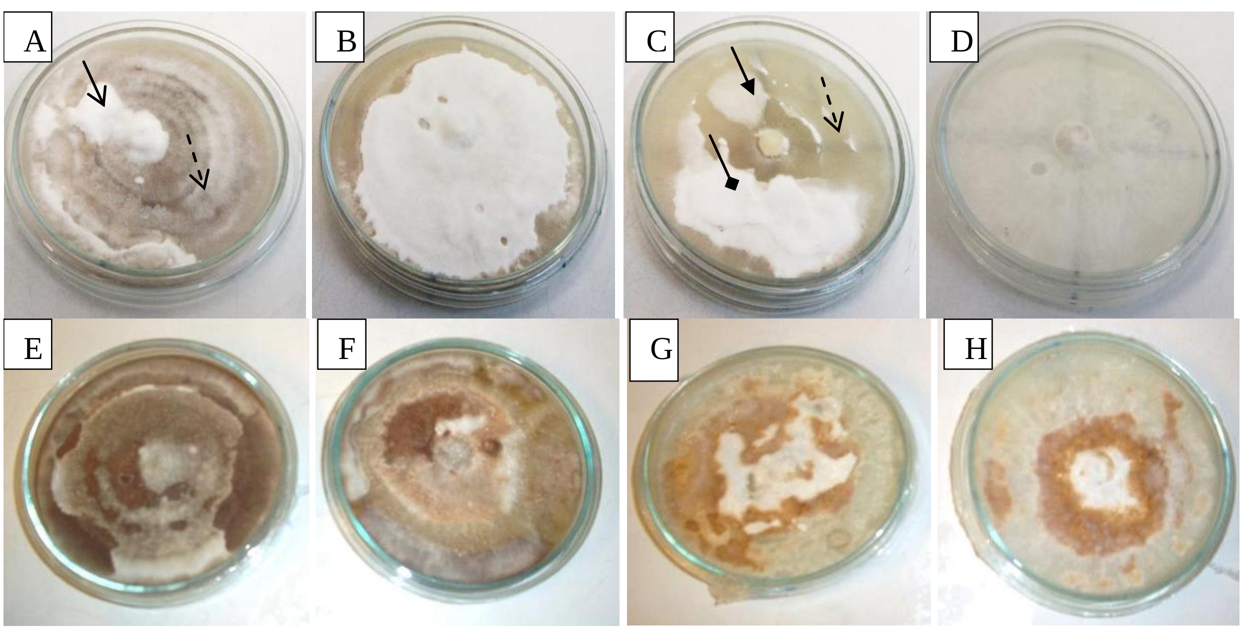

Figure 5. *Lentinus squarrosulus* strains *Sqw* and *Lsf* (top and bottom rows respectively) colony morphology and mycelia density on the 26th day of incubation ("aged" cultures) at 25 ℃ on various solid media. A and E, B and F, C and G, and D and H represent the strains inoculated on EG, RS, TH, and SD respectively. Arrows point to some observed sectors of mycelia colonies on the media.

The culture morphologies on the various substrates changed upon further incubation (or with maturity) for both strains. This was clearly apparent by the 26th day of incubation (Figure 5). Though the concentric rings observed for *L*. *squarrosulus* strain *Sqw* on the 7th day of incubation on EG remained prominently present with age (Figure 4A and 5A), that on RS and TH were not obvious media at both the initial and later stages of incubation, it appears reasonable to infer that *L. squarrosulus* culture morphology on solid media is strain dependent and that pigmentation of the mycelia or cultures of the strains occurs differently on the media with extended incubation.

by the 26th day of incubation (Figure 4B and 4D and (Figure 5B and 5D respectively). On the 26th day of incubation, the mycelia of *L*. *squarrosulus* strain *Sqw* on EG appeared polymorphic (Figure 5A). This polymorphism was in the form of sectoring and difference in mycelia coloration. For instance, the sectors with cottony mycelia were whitish (Figure 5A; solid arrow) whereas the longitudinally radial mycelia had brownish coloration. Furthermore, there were regions on this substrate (EG) where the brownish coloration had a layer of whitish mycelia over it (Figure 5A; dashed arrow). On RS, virtually the whole *L*. *squarrosulus* strain *Sqw* mycelia turned densely white, forming a dense mat (Figure 5B), rather than the longitudinally radial mycelia observed at the initial stages of incubation, by the 26th day of incubation. Strain *Sqw* also showed polymorphism with age on TH, on which the mycelia showed undefined sectors of densely white cottony mycelia, densely white mat of mycelia, and sectors with low mycelia density by the 26th day of incubation (Figure 5C; solid, diamond, and dashed arrows respectively). The only difference observed on SD by the 26th day of incubation was the improved mycelia density (Figure 5D). The mycelia remained uniformly white with longitudinally radial mycelia on this solid media (Figure 5D). It is worth noting that the strain only changed a colour change when cultured on EG.

Culture morphology of *L. squarrosulus* strain Lsf

Variations in culture morphology of the strain on the various solid media under the study conditions were eminent both at the initial incubation stage and with extended incubation (Figure 4 and 5E, F, G and H). While the strain showed longitudinally radial morphology on all the solid media studied by the 7th day of incubation (Figure 4E, F, G and H), concentric rings were absent on EG and SD (Figure 4E and H respectively) whereas there was the presence of concentric rings when cultured on RS and TH (Figure 4F and G respectively).

With extended incubation of the strain, polymorphisms were apparent on all the solid media (Figure 4E, F, G and H). As indicated for *Sqw*, the observed polymorphisms appeared in the form of sectoring and difference in mycelia coloration. The colours ranged from white to shades of brown (Figure 4E, F, G and H). Occurrence of different mycelia morphology in terms of presence or absence of concentric rings, and colour and density of mycelia has also been reported on *L. cladopus* Lév (Atri and Guleria, 2013). Comparing the culture morphologies of the 2 strains presently studied on the various solid However, this is also dependent on the strain inoculated. *Polyporus arcularius* mycelia also show a change from white, dense aerial mycelia to light brown crusts of mycelia with further incubation on Y2 solid media (Hibbett et al., 1993). Changes in colour of five *Lentinus* spp. including *L. squarrosulus* cultures from white to brown mycelia during incubation on PDA has also been reported (Sharma and Atri, 2013). Contrary to the report by Sharma and Atri (2013), the changes in mycelia colour on the solid media in this study did not occur from the middle of the plate, but rather, randomly. The authors of the said manuscript attributed the specific changes in the culture morphology to the species under study. However, based on the results of the present study, we infer that *Lentinus spp.* culture morphology also depends on the strain under study and on the growth media used.

According to Stamets and Chilton (1983), a mycelium is senescent when it grows old. The authors have indicated that signs of senescing include a change from rhizomorphic to cottony looking mycelia. Thus, it can be inferred that both sectoring and pigmentation could be signs of senescing in *L. squarrosulus* cultures.

However, based on these studies, it cannot be clearly deduced whether the pigmentation observed in the 26 day old cultures of *Lsf* is solely genetic and what the composition of the observed pigments are. It is also unclear whether this pigmentation is as a result of enzymatic browning caused by enzymes released during incubation (Tripathi et al., 2012; Wuyep et al., 2003) and the phenolic compounds present in the mycelia (Mhd Omar et al., 2011). Further studies are required to investigate these observations and to discover biotechnological applications of enzymes or compounds present in *Lsf* with extended incubation on solid media. The effect of the mycelia morphology on the quality of spawns produced with its consequent effect on fruit body yield will be investigated in further studies.

Relationship between mycelia growth rate and mycelia density

Various degrees of negative correlation were obtained between mycelia growth and the mycelia densities observed on the 7th and 26th days of incubation (Table 1) for strain *Sqw*. This was observed throughout the incubation period, regardless of the incubation time. This observation was obvious on EG, for instance, on which although the mycelia growth rate was lowest (0.47 cm/day), the mycelia densities of both the young and "aged" cultures (Figure 3 and 4 top rows respectively) were relatively high. Conversely, a strong positive correlation (0.96; Table) was obtained between the mycelia densities observed on the 7^{th} and 26^{th} days of incubation on the various solid media under the study conditions. This same trend was observed for strain *Lsf*. These results indicate that *L. squarrosulus* mycelia

Table 1. Table of correlations between mycelia extension during incubation and mycelia densities on days 7 and 26 of incubation for strain *Sqw*. A value closer to +1 means there is a high positive correlation, a value closer to -1 shows a strong negative correlation and a value of 0 means there is no correlation between compared parameters.

Correlation						
Day_3						
Day_4	0.8762					
Day_5	0.7434	0.8592				
Day_6	0.7429	0.8829	0.8772			
Day_7	0.7336	0.8414	0.9315	0.9149		
Mycelia_density_day_7	-0.5181	-0.6161	-0.8063	0.6919	-0.8482	
Mycelia_density_day_26	-0.4493	-0.4999	-0.7478	0.5774	-0.7677	0.9621
	Day_3	Day_4	Day_5	Day_6	Day_7	Mycelia_density_day_7

density is not dependent on the growth rate and vise-versa, irrespective of the strain. Thus, a strain that is growing fast on a given media does not necessarily have to have dense mycelia. However, mycelia having a high density at the beginning of incubation are more likely to have a corresponding high mycelia density at the end of incubation.

Conclusion

Mycelia growth rate, density and morphology of *L. squarrosulus* is media and strain-dependent. Cultures of the mushroom differ morphologically between the strains studied and on the different solid media. These differences are more pronounce with extended incubation. Irrespective of the strain, *L. squarrosulus* mycelia density is not dependent on the growth rate, and vise-versa.

Conflict of Interests

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

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Azosprillium based integrated nutrient management for conserving soil moisture and increasing sorghum productivity

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In this field study, we evaluated the utilization of *Azosprillium* with organic amendments and urea in winter sorghum productivity in order to achieve the best results with minimum nitrogen utilization in Vertisols under different rainfall situations of SAT, India. Results showed that the organic materials application conserved more rainwater in top 0.60 m soil profile. Across three years of study, *Azospirillum* seed inoculation had a positive effect on sorghum production and produced 13% more sorghum grain and straw yields compared to 0 kg N ha^{-1}. Sorghum grain yields were 44% higher when seeds were treated with *Azospirillum* and applied with 50% recommended rate of nitrogen (RRN) through urea and 50% RRN through organic materials (50:50 ratio of *Leucaena* loppings and farm yard manure) compared to control. Water use efficiency (WUE) was 42% greater with *Azospirillum* seed treatment and application 50% RRN through organic materials and 100% RRN through urea over control. Interference drawn in this study clearly indicates that low cost technology of *Azospirillum* seed treatment has to be adopted for higher sorghum grain yields. However, it is recommended to apply N through organic and fertilizer N in the ratio of 50:50 RRN along with *Azospirillum* seed treatment for sustainable sorghum yields and greater water productivity in the Vertisols of SAT, India.

Key words: *Azospirillum,* nitrogen, rainwater conservation, Vertisols, winter.

INTRODUCTION

The global human population will reach around 10 billion by 2050. To meet the needs of growing population, the pressure on the reduced land area will increase to produce more food, fibre and fuel by greater use high yielding crop cultivars, fertilizers and employing new production technologies at farm level (Johri et al., 2003). In India, nearly 60% of cultivated area is rainfed that contributes for 45% of agricultural production (Venkateswarlu, 2008a, b). Vertisols are major soils in rainfed eco–system of SAT in south India. These soils are sometimes more hungry than thirsty as low soil fertility is one of the major factor limiting crop productivity. These Vertisols are universally deficient in N and nearly 40% in P thus requires proper management to sustain productivity. Low rainfall (508 mm average of 56 years) and its uneven distribution; poor economic status of

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dryland farmers, high costs and timely unavailability of fertilizers and organic amendments in recent years resulting in lesser application than the recommended rate. This practice leads to higher rate of nutrients depletion than their replenishment that declines the soil health and consequently reduces crop productivity over the years (Math and Patil, 2013). Decreased availability of organic amendments, increased cost of chemical fertilizers and organic amendments farmers are forced to pay nearly double the cost of fertilizers during 2012 to 2013 alone in India. Frequent droughts due to greater variation in rainfall as a result of climate change resulting in higher cost of production and decreased net returns per unit area. Adverse effect of water stress on productivity of rainfed crops especially during drought years can be minimized by use of organic materials as soil amendments. These amendments conserve rainwater in situ; enhance nutrients supply and increase N use efficiency of applied fertilizers (Reddy et al., 2004; Patil and Sheelavantar, 2009). To meet crop nutrient requirements exclusively through organic amendments alone requires large quantity of materials which is uneconomical due to its high cost and low availability. Application of chemical fertilizers alone does not bring significant increase in crop yields on sustainable basis over the years as these fertilizers supply only limited nutrients.

In addition, continued use of chemical fertilizers causes health and environmental hazards such as ground and surface water pollution by nitrate leaching. So reducing the amount of nitrogen fertilizers applied to the field without a nitrogen deficiency will be the main challenge in field management. One of the possible options to reduce the use of chemical fertilizer could be using of organic materials. It is generally acknowledged that organic materials play an important role in maintaining a high level of soil fertility. The positive influence of organic fertilizers on soil fertility, on crop yield and quality has been demonstrated in the works of many researchers (Suresh et al., 2004; Naeem et al., 2006; Dauda et al., 2009). Increase in price of chemical fertilizers and organic amendments forcing the farmers in the region to use alternative low cost inputs like biofertilizers.

Biofertilizers play an important role in plant nutrient management, particularly in rainfeds where farmers wants to reduce the fertilizer application through biofertilizers that costs them low. Applications of biofertilizers improves soil organic matter content, enzymes, microbial population and decrease the negative effect of chemical fertilizer and also increase yields of crops on sustainable basis (Alizadeh and Ordookhani, 2011; Jala-Abadi et al., 2012). Among the plant growth promoting *rhizobacterium* (PGPR) species, *Azospirillum* spp. is well known for its ability to excrete. Earlier research indicates that cereal crops in general, and sorghum and pearlmillet in particular have a variety of N fixing bacteria in its rhizosphere, including *Azospirillum* and *Azotobacter* which releases growth promoting substances like phytohormones such as indole acetic acid, gibberellins, cytokinins and auxins. For instance, these phytohormones fixes nearly about 10 to 25 kg N ha^{-1} thus increasing root biomass and grain yield (Kumar and Gautam, 2004; Fuentes-Ramirez and Caballero-Mellado, 2005).

In view of the present existing situation,it is more essential to adopt Integrated plant nutrient supply system (IPNS) system for sustainable crop productivity by the farmers who have greater concerns on soil health. Under IPNS, balanced use of organic amendments along with chemical fertilizers including micronutrients and biofertilizers results in improved soil fertility and crop productivity through fertilizer use efficiency (Singh et al., 2004; Guggari and Kalghatagi, 2005; Jala-Abadi et al., 2012).

Sorghum is one of the major rainfed cereal crop cultivated in 41 Mha that produces 64.20 Mt, with a productivity hovering around 1.6 tons per ha. Unlike in other parts of world, sorghum is cultivated in both rainy and postrainy seasons in India. In India sorghum is cultivated in 7.69 Mha that produces around 7.29 Mt with a low productivity of 948 kg ha^{-1} (Tonapi et al., 2011). Quality of rainy season sorghum is poor and is mainly used for animal and poultry consumption. Postrainy season sorghum grain is mostly consumed by human beings and is a staple food of dryland dwellers in central and south India. Sorghum is cultivated on stored soil water in profile during winter season. Deficit soil moisture and low availability of required quantity of nutrients at critical crop growth stages of sorghum reduces crop yields in Vertisols of Deccan Plateau region in India. To enhance winter sorghum productivity, various agronomic management practices have been designed and evaluated, however the information on IPNS with biofertilizers is scarce under different rainfall situations.

In view of this situation, the present field study aims to explore the effect of bio-organic and organic materials and chemical fertilizers on rainwater conservation, water use efficiency and sorghum productivity during winter season.

MATERIALS AND METHODS

Soil and site characteristics

A field study was conducted for three years (2000 to 2001 to 2002 to 2003) at CSWCRTI's Research farm, Bellary, India (15°09' N latitude, 76°51' E longitude, and at an altitude of 445 m above msl). Experimental soils were classified as Typic–Pellusterts of Bellary series derived from granite, gneiss and schist. Clay content increased with depth from 44% on surface to 50% at 0.90 m. Infiltration rate of these soils is 0.8 mm h^{-1} with bulk density of 1.25 Mg m^{-3} (Black, 1965). These soils pH is 8.6, electrical conductivity is 0.14 dS m^{-1} and organic carbon content is 3.83 g kg^{-1} (Piper, 1966). The available N of these soils is low 159 kg ha^{-1} (Subbaiah and Asija, 1956), medium available P of 21 kg as P_2O_5 ha^{-1} (Jackson, 1967) and high available K of 550 kg as K_2O ha^{-1} (Muhr et al., 1965). Chickpea was cultivated at study site during 1999 to 2000.

Table 1. Nutrient content of organic materials.

Organic materials	N (%)	P (%)	K (%)
Leucaena loppings	2.50	0.79	1.50
Farmyard manure	0.66	0.38	0.84

Treatment details

A field study was conducted with 10 treatments combinations of N applied through organic materials, urea and PGPR *Azospirillum brasilense.* The treatments details includes, that is, T_1–0 kg RRN ha^{-1} (Control), T_2–*Azospirillum*, T_3–50% recommended rate of nitrogen (RRN) through organic material, T_4–50 RRN through organic material + *Azospirillum,* T_5–50% RRN through urea, T_6–50% RRN through urea + *Azospirillum,* T_7–100% RRN through urea, T_8–100% RRN through urea + *Azospirillum,* T_9–50% RRN through urea +50% RRN through organic material + *Azospirillum* and T_{10}–100% RRN through urea + 50% RRN through organic material + *Azospirillum.* This study is conducted in a randomized block design with three replications. The organic N is applied through Leucaena loppings and farmyard manure (50% each) on N content basis (Table 1). Leucaena loppings and farmyard manure were incorporated in soil during second and third week of August, respectively in top 10 cm soil depth as per the treatments. Seeds were treated with PGPR *A. brasilense* prior to sowing and N was applied through urea as per treatments at sowing along with recommended rate of P_2O_5, that is, 40 kg ha^{-1}. Sorghum (*Sorghum bicolor* (L.) Moench) during winter season was sown at 5 cm depth with seeds spaced at 15 and 60 cm in rows apart during 2000 to 2001 and 2001 to 2002, while during 2002 to 2003; sorghum was sown at 5 cm depth with plant to plant spaced at 17.5 cm in 60 cm rows apart. During 2000 to 2001, sorghum cultivar SPV–86 and during 2001 to 2002 and 2002 to 2003 sorghum cultivar Maldandi (M35–1) were sown. Each smallest plot measured 5.4 m wide and 6.0 m long. At harvest, head and straw from individual plots were harvested; sun dried for 10 days and after separation of grains from head; the grain and straw weights were recorded.

Computation of soil water and water use efficiency

Profile soil moisture was gravimetrically recorded at every 0.15 m soil depth up to 0.60 m soil depths in each treatment prior to sowing and at 30, 60 and 90 days after sowing (DAS) and at harvest. Soil moisture utilized was computed as the difference of soil moisture at sowing, at 30, 60 and 90 DAS and at harvest. Consumptive use of water was determined by recording difference in values of soil moisture content (mm) in top 0.60 m of soil between any two stages, by adding rainfall and subtracting runoff during the relevant period (Patil, 2013; Patil and Sheelavantar, 2006). No drainage or deep percolation was observed at Bellary during the crop growth period and hence it was not accounted for calculation of consumptive use of water during three years of study period. Runoff that was measured by using multi–slot device from adjacent study plot was used for this study in 10 treatments of 3 replications, that is, total 30 treatments for assessing each treatment runoff. Difference in soil water was added to arrive at consumptive use of water in mm by using the formula,

$$CUW = \sum_{i=1}^{n} (SM_a - SM_b) + \text{Rainfall} - \text{Runoff},$$

Where CUW = Consumptive use of water (mm),
SM_a = Soil moisture content in top 0.60 m soil at stage a,
SM_b = Soil moisture content in top 0.60 m soil at stage b,
Rainfall and Runoff as recorded between stage a and b. The stages a and b are sowing, 30 days after sowing (DAS), 60 DAS, 90 DAS and harvest.

The economic sorghum yield, i.e., grain yield was divided by CUW (mm) to work out the water use efficiency (WUE). The WUE is expressed in kg ha^{-1} mm^{-1}.

Growth and yield components of sorghum

Average plant height (base to the tip of the head) is computed from five randomly selected plants from each plot and expressed in meter. Biometric characterization of sorghum head was computed by measuring head length (base to tip) and head girth (perimeter at the centre of the head) of five randomly selected plants from each plot and expressed in centimeter. Weight of the head and grain yield per plant were recorded after drying for 10 days. The sample of 1000 grains were randomly drawn from individual treatment in all three replications were weighed and expressed in grams. The grains per head was estimated using the formulae,

$$\text{Number of grains per head} = \frac{\text{Grains weight per head}}{\text{1000–grains weight}} \times 1000$$

The Harvest index (HI) is computed using the formulae (Donald 1962).

$$\text{Harvest index} = \frac{\text{Economic yield}}{\text{Total biological yield}} \times 100$$

Statistical analysis

All the field data in this study was analyzed using MSTAT–C statistical package (Gomez and Gomez, 1984). When analysis of variance indicated significant difference, LSD test was used for comparing the differences between the treatments.

RESULTS AND DISCUSSION

Years

During 2001 the rainfall of 566.6 mm that fell in 31 rainy days was 12% higher. In addition, the antecedent rainfall of 184.0 mm that fell from 16 to 25 September 2001 resulted in timely sowing of winter sorghum on 27 September 2001 with uniform wetting of top 60 cm soil depth at sowing. Apart from timely sowing, uniform distribution of 248.5 mm rainfall in 14 rainy days during cropping season resulting in higher soil moisture in profile from sowing till physiological maturity thus produced better plant growth and increased the winter sorghum grain yields by nearly 70 and 53% during 2001 to 2002 compared to 2002 to 2003 and 2000 to 2001, respectively (Figure 1, Tables 2 and 3). Ghanbari et al. (2013) also reported increased maize yield with application of PGPR rhizobacteria and Zinc under favourable soil moisture as compared to stress situations. Late sowing of sorghum on 13 and 21 October with lower crop season rainfall of 45.6 and 9.8 mm that fell during 2000 to 2001 and 2002 to 2003, respectively produced lower yields. Higher grain yield during 2001 to 2002 compared to 2000 to 2001 and 2002 to 2003 was attributed to greater head weight, grain weight per plant, 1000 grains weight and greater head length and head girth (Table 4). Even the trend in straw production was similar to grain and is attributed to production of taller plants with more leaves higher dry matter production in both leaves and stem (Table 4). More dry matter translocation from leaves and stem to head at maturity during 2000 to 2001 produced higher HI by 15 and 24% compared to 2001 to 2002 and 2002 to 2003, respectively. The harvest index in maize was greater under favorable soil moisture conditions and decreased with water deficit stress conditions (Hagbabayi et al., 2011; Ghanbari et al., 2013). Lower consumptive use with more grain production per each unit of water utilized during 2000 to 2001 produced greater WUE of 8.53 kg ha^{-1} mm^{-1} and it was higher by 23 and 14% compared to 2002 to 2003 and 2001 to 2002, respectively (Table 4). In arid and semi arid regions abiotic stresses such as water stress, salinity, high light, temperature, flooding, toxic metals, wounding and biotic stresses including pests and pathogens reduced plant growth and yield (Glick et al., 2007; Ghanbari et al., 2013). Grain yield of maize reduced due to water stress (Sajedi et al., 2009; Habibi et al., 2010; Farajzadeh et al., 2011; Ghanbari et al., 2013).

Treatments

Azospirillum seed treatment produced 7% more grain yield compared to control during 2001 to 2002 with higher rainfall and its uniform distribution. During 2000 to 2001 with a higher rainfall of 550.9 mm and its uneven distribution, sorghum grain yield was higher by 13%, while in a drought year with lower annual rainfall of 432.6 mm during 2002 the sorghum grain yield was 23% higher with *Azospirillum* seed treatment compared to 0 kg N ha^{-1} during 2002 to 2003. Across three years, sorghum grain and straw yields were higher by 13% with *Azospirillum* seed treatment over control (Table 4). These results indicate that a simple low cost technology of seed treatment with *Azospirillum* is more beneficial during drought years with less soil water availability at different stages of sorghum compared to normal and higher rainfall years. Seed inoculation of wheat varieties with biofertilizers showed a significantly increased vegetative growth and grain yield (Narula et al., 2001; Jala-Abadi et al., 2012). Higher sorghum yields with *Azospirillum* inoculation was probably attributed to the biosynthesis and secretion of bacterial Indole–3–Acetic Acid (IAA) (Johri et al., 2003; Fuentes-Ramirez and Caballero-Mellado, 2005). Bacterial IAA stimulates plant root production, especially lateral roots with greater root hairs and improves their development (Meunchang et al., 2004). Okon and Kapulnik (1986) also pointed out that colonization of plant root by *Azospirillum* might enhance permeability of N, P, and K ions into roots. The plant growth promoting *rhizobacterium* (PGPR) enhances uptake of soil nutrients by host plant (Salamone et al., 1997; Fuentes-Ramirez and Caballero-Mellado, 2005). Higher grain yield of winter sorghum with *Azospirillum* inoculation compared to 0 kg N ha^{-1} was attributed to production of greater dry matter in head due to greater head and grain weight per plant, higher values of 1000 grains weight and higher head length and head girth (Table 4).

The sorghum grain yield enhanced from 13 to 38% with application of 50% RRN through organic material along with *Azospirillum* (T_4), while increase was higher and varied from 33 to 47% with application of 50% RRN through urea along with *Azospirillum* (T_6) during different years of study. Further, increase in N application from 50% to 100% through urea along with *Azospirillum* (T_8) increased grain yield marginally compared to control during all the three years of study (Table 3 and Figure 1). In pooled data, mean grain yield of three years in T_8 was 40% higher compared to control (Reddy and Sudhakarbabu, 1996). In addition to N fixation, *Azospirillum* improves plant growth through production of phytohormones leading to better plant growth with greater photosynthate production and its translocation to reproductive parts (Fuentes-Ramirez and Caballero-Mellado, 2005; Arbad et al., 2008; Prasad, 2008; Khan et al., 2010). Higher head and grain weight per plant with higher values of head length, head girth and 1000–grains weight produced higher grain yield in T_4, T_6 and T_8 treatments compared to control during all three years of this study. Application of bacterial inoculants at higher soil moisture with zinc sulphate increased maize cob weight (Ghoorchiyani et al., 2011; Mostafavi et al., 2012; Ghazvineh and Yousefi, 2012).

Applying 50% RRN through urea and 50% RRN through organic materials and seed treatment with *Azospirillum* (T_9) produced significantly higher grain yield varying from 32 to 66%, while increase in N application through urea to 100% (T_{10}), sorghum grain yield was 37 to 67% higher compared to 0 kg N ha^{-1} during study period and in pooled data (Table 3 and Figure 1). These results indicates that *Azospirillum* seed inoculation fixes around 10–25 kg N ha^{-1} and could save nearly 50% of recommended rate of N through urea in winter sorghum (Prasad, 2008). Occurrence of moisture stress from flower initiation to maturity due low soil water availability in profile and higher N through urea negatively interacted during a drought year of 2002 to 2003 thus marginally

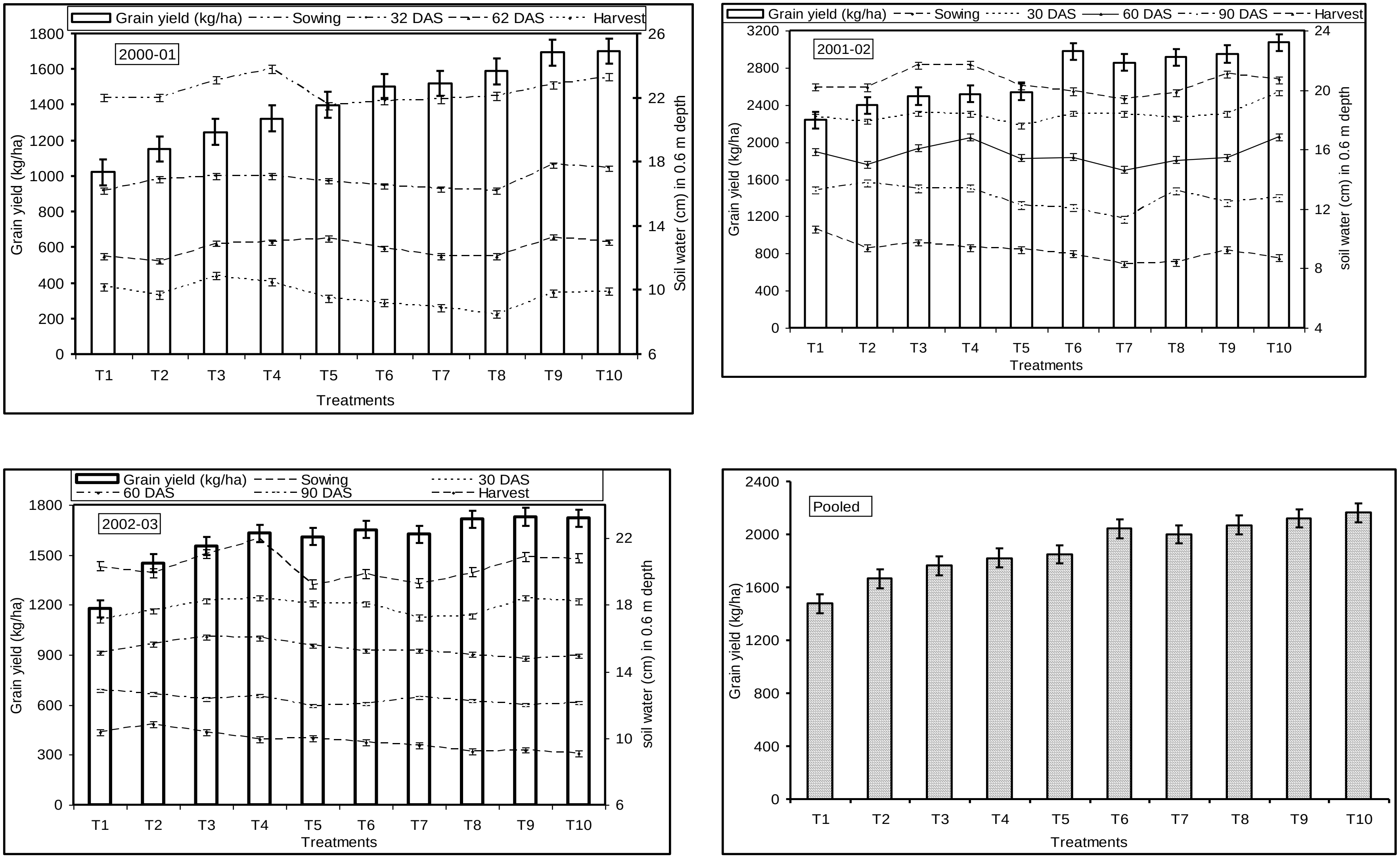

Figure 1. Grain yield (kg ha^{-1}) and soil water (cm) in 0.60 m soil profile as influenced by integrated nutrient supply system in winter sorghum in Vertisols of Bellary, India.

Table 2. Annual rainfall (mm), crop season rainfall and antecedent rainfall, number of rainy days during different years, date of sowing and date of harvest of sorghum.

Particulars	Years		
	2000 to 2001	2001 to 2002	2002 to 2003
Annual rainfall (mm)	550.9	566.6	432.6
Crop season rainfall (mm)	45.6	253.5	9.8
Antecedent rainfall	187.1 (3 to 12 October)	184.0 (15 to 25 September)	122.7 (8 to 17 October)
Number of rainy days during the year	34	31	32
Number of rainy days during crop season	3	14	1
Date of sowing	13.10.2000	25.09.2001	21.10.2002
Date of harvest	13.02.2001	30.01.2002	28.02.2003
Classification of years	Above normal year with uneven distribution	Above normal year with uniform distribution	Drought year

Table 3. Grain yield, straw yield, harvest index, water use efficiency and plant height of winter sorghum as influenced by integrated plant nutrient supply system.

Treatment	Grain yield (kg ha^{-1})			Straw yield (t ha^{-1})			Harvest Index (%)			WUE (kg ha^{-1} mm^{-1})			Plant height (m)		
	2000 to 2001	2001 to 2002	2002 to 2003	2000 to 2001	2001 to 2002	2002 to 2003	2000 to 2001	2001 to 2002	2002 to 2003	2000 to 2001	2001 to 2002	2002 to 2003	2000 to 2001	2001 to 2002	2002 to 2003
Year	1414	2693	1586	2.06	4.91	3.23	40.6	35.4	32.8	8.53	6.95	7.46	1.61	1.87	1.66
LSD (P< 0.05)		147			0.25			0.5			0.72			0.06	
T_1–0 kg RRN ha^{-1}	1020	2234	1176	1.62	4.15	2.52	38.8	34.9	31.8	6.94	5.81	5.34	1.47	1.71	1.54
T_2–*Azospirillum*	1152	2395	1449	1.70	4.67	3.02	40.3	33.9	32.4	7.11	6.23	6.95	1.49	1.77	1.62
T_3– 50% RRN through organic material	1246	2493	1553	1.82	4.67	3.18	40.8	34.9	32.9	7.74	6.23	7.10	1.54	1.79	1.62
T_4–50 RRN through organic material + *Azospirillum*	1323	2516	1629	2.33	4.85	3.40	35.9	34.1	32.4	7.69	6.29	7.98	1.59	1.87	1.63
T_5–50% RRN through urea	1398	2539	1609	1.99	4.84	3.30	41.3	33.0	32.9	8.79	6.58	7.95	1.62	1.79	1.66
T_6–50% RRN through urea + *Azospirillum*	1502	2976	1652	2.04	5.05	3.40	42.3	37.1	32.7	9.10	7.77	7.95	1.66	1.95	1.70
T_7–100% RRN through urea	1520	2854	1623	2.04	4.97	3.05	42.5	36.5	34.8	8.79	7.58	8.03	1.66	1.85	1.68
T_8–100% RRN through urea + *Azospirillum*	1588	2910	1714	2.21	5.19	3.33	41.8	36.1	34.1	9.29	7.64	8.21	1.68	1.94	1.70
T_9–50% RRN through urea +50% RRN through organic material + *Azospirillum*	1694	2945	1728	2.21	4.92	3.62	43.4	37.5	32.4	10.02	7.48	7.91	1.69	1.95	1.74
T_{10}–100% RRN through urea+ 50% RRN through organic material + *Azospirillum*	1701	3071	1719	2.61	5.49	3.50	39.5	36.0	32.9	9.83	7.88	7.91	1.71	2.04	1.70
LSD (P< 0.05)	376	254	176	0.50	0.66	0.37	n.s.	n.s.	2.5	0.79	0.65	0.81	0.13	0.18	n.s.

decreased sorghum grain yields from 1728 kg ha^{-1} under T_9 to 1719 kg ha^{-1} in T_{10}.

Straw yield during study period and in pooled data was higher and it varied from 32 to 61% in T_{10} compared to control (Table 3 and Figure 1). Higher grain and straw yield in T_9 and T_{10} were attributed to more rainwater conservation and its availability in soil profile from sowing to 60 days after sowing and increased the use efficiency of applied N fertilizer either through organic/inorganic materials/*Azospirillum* (Figure 1). Higher soil water and nutrient availability at different stages of sorghum produced better plant growth with greater dry matter translocation to head from leaf and stem during physiological maturity thus

Table 4. Yield components of winter sorghum as influenced by integrated plant nutrient supply system.

Treatment	Head weight (g plant^{-1})			Grain weight (g plant^{-1})			1000-Grain weight (g)			Head length (cm)			Head diameter (cm)		
	2000 to 2001	2001 to 2002	2002 to 2003	2000 to 2001	2001 to 2002	2002 to 2003	2000 to 2001	2001 to 2002	2002 to 2003	2000 to 2001	2001 to 2002	2002 to 2003	2000 to 2001	2001 to 2002	2002 to 2003
Year	34.65	47.90	41.00	28.48	40.71	29.72	26.34	32.28	27.11	14.4	16.9	16.5	13.7	13.7	14.0
LSD (P< 0.05)		5.87			4.15			1.82			0.60			n.s.	
T_1–0 kg RRN ha^{-1}	29.67	42.33	36.33	23.50	34.90	24.59	23.90	29.36	24.73	12.9	14.9	14.8	12.7	12.0	12.2
T_2–*Azospirillum*	31.33	44.00	37.00	24.90	35.91	25.91	24.70	30.60	26.21	13.4	15.7	15.2	12.2	12.9	13.3
T_3–50% RRN through organic material	31.70	45.00	37.67	25.40	36.90	27.07	24.76	31.40	26.57	14.0	16.3	15.8	12.4	13.0	13.9
T_4–50 RRN through organic material + *Azospirillum*	32.67	46.67	39.33	26.40	38.13	26.89	25.41	31.77	27.57	13.9	16.6	16.7	12.8	13.4	14.2
T_5–50% RRN through urea	33.33	47.33	39.67	27.00	41.54	30.62	25.92	31.89	26.31	14.2	16.8	16.7	13.0	13.6	14.2
T_6–50% RRN through urea + *Azospirillum*	36.07	51.33	41.00	29.40	43.94	30.98	27.06	34.37	28.04	14.3	17.7	17.0	14.1	14.0	14.8
T_7–100% RRN through urea	36.70	50.67	42.67	30.70	44.01	32.41	27.39	33.39	26.81	14.8	17.3	16.7	14.5	14.1	14.6
T_8–100% RRN through urea + *Azospirillum*	37.33	50.00	45.00	30.93	43.13	33.04	27.79	32.26	27.36	15.1	17.2	17.1	14.8	14.3	14.5
T_9–50% RRN through urea +50% RRN through organic material + *Azospirillum*	38.70	50.67	47.00	32.73	43.96	32.41	28.22	33.13	29.00	15.7	17.9	17.5	15.1	13.9	14.7
T_{10}–100% RRN through urea+ 50% RRN through organic material + *Azospirillum*	39.00	51.00	44.33	33.83	44.64	33.33	28.29	34.62	28.51	16.0	18.0	17.2	15.4	14.7	14.4
LSD (P< 0.05)	5.73	n.s.	6.37	6.07	5.87	5.09	2.72	1.85	2.24	n.s.	1.61	0.97	2.24	1.40	1.41

producing greater head weight plant^{-1}, grain weight plant $^{-1}$, 1000–grains weight, head length and diameter in T_9 and T_{10} (Kundu et al., 2009;Thakur et al., 2009). Greater grain and straw yields in T_9 and T_{10} were also attributed to balanced supply of nutrients through organic and inorganic materials with increased N use efficiency as reported earlier by Singh and Agarwal (2005), Kumar et al. (2005), Laddha et al. (2006) and Jala-Abdi et al. (2012). In Vertisols of Bijapur, India, adoption of moisture conservation practices with increased N application up to 50 kg ha^{-1} produced greater dry matter accumulation plant^{-1} resulting in higher grain and straw yield in sorghum (Math and Patil, 2013; Patil et al., 2011). Application of farmyard manure at 5 t ha^{-1} and 30 kg N ha^{-1} along with *Azospirillum* seed inoculation resulted in greater pearlmillet yield over other treatments as reported by Kumar and Gautam (2004). Results of this study clearly indicate that N application through either organic, inorganic or microbial cultures alone were not beneficial compared to their combination in increasing cereals yields and was attributed to soil and rainwater conservation and supply of all nutrients at required rates at different sorghum growth stages. In corn and sorghum also biological fertilizer was not sufficient but integrated application of biological and chemical fertilizers produced significant increase in yield (Amujoyegbe et al., 2007; Rizwan et al., 2008; Haghighi et al., 2010).

Greater dry matter translocation from leaves and stem to head at physiological maturity producing 7% higher HI with application of 50% RRN through urea, 50% RRN through organic materials and *Azospirillum* seed treatment compared to control during all three years of study and in the pooled data. Conserved rainwater in soil profile was better utilized for grain production thus producing higher WUE during 2000–01 compared to 2001 to 2002 and 2002 to 2003. In pooled data, WUE was 12% higher with *Azospirillum* seed inoculation over control. The WUE across three years of study increased from 16% (T_3) to 21% (T_4), 29% (T_5) to 37% (T_6) and 35% (T_7) to 39% (T_8) with *Azospirillum* seed treatment compared to application of organic or inorganic materials at same rate. The water use efficiency (WUE) was also greater by 41 and 42% with application of 50% RRN through urea, 50% RRN through organic materials and seed treatment with *Azospirillum* (T_9) and application of 100% RRN through urea, 50% RRN through organic materials with *Azospirillum* seed treatment (T_{10}), respectively compared to control (Table 3 and Figure 1). Higher WUE with urea alone compared to application of same quantity of N through organic materials or in combination of urea and organic materials was attributed to lower consumptive use of water with application of urea alone. Organic materials conserved more rainfall with higher soil water in profile resulting in higher consumptive use with lower WUE.

Conclusions

Organic materials conserved rainwater in-situ. *Azospirillum* seed inoculation produced 13% greater sorghum yields and was economically more beneficial during drought years compared to normal rainfall situations. Poor farmers with animal components apply available organic amendments with low cost *Azospirillum* for greater winter sorghum yields. Resourceful farmers apply available organic amendments at farm along with 50% RRN through urea during normal rainfall years and top dress 20 kg N ha^{-1} with urea during October second fortnight during above normal rainfall situations with better soil fertility and sustained sorghum yields in Vertisols of SAT, India. *Azospirillum* seed inoculation alone increased the WUE by 12% and further integrated nutrient management improved the water productivity in winter sorghum up to 41%.

Conflict of Interests

The author(s) have not declared any conflict of interests

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Review

Importance of designer eggs for the Nigerian population

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Epidemiological studies have led to recommendations that people should consume at least two servings of fruit and three servings of vegetable daily for healthy living but majority of Nigerians falls well short of meeting these guidelines. Hence, there is a need to make up for this shortage. Through studies, it has been established that in addition to being a natural functional food, the egg's nutrient content can be altered by designing the feed given to the chickens thus resulting in the production of designer eggs. A crucial feature of these designer eggs is the synergistic combination of healthy Omega-3 fatty acids with major antioxidants, Vitamin E and lutein, as an important approach to the improvement of the human diet. These eggs will not be able to replace vegetable and fruits as a major source of natural antioxidants and fish products but can substantially improve the diet, especially in a country like Nigeria, significantly contributing to the recommended daily intake of essential nutrients. Thus, this study reviews the importance of designer eggs in the Nigerian context.

Key words: Vitamin E, designer egg, omega-3 fatty acids, antioxidants, lutein, Nigeria.

INTRODUCTION

Eggs have been described as "Nature's original functional food" (Hasler, 2000) packed with various important vitamins and minerals. Eggs are said to contain the highest quality protein, when compared to other animal protein sources and they are inexpensive when compare to other protein sources.

Chicken's eggs have been used as a food by human beings since antiquity. Compared with the hen's egg, no other single food of animal origin is eaten by so many people all over the world and none is served in such a variety of ways. Its popularity is justified not only because it is so easily produced and has so many uses in cookery, but also because of its nutritive excellence.

The nutrient value of one egg have been tabulated in Table 1 and per the World Health Organization (WHO) recommendation, 2 eggs per day are required for optimum growth.

Eggs contain a number of beneficial nutrients, some of which have functions that are currently being studied. Egg yolks provide an excellent, highly bio-available source of the carotenoids, lutein and zeaxanthin (Handelman et al., 1999). Recent research demonstrated the link between these dietary compounds and the macular pigment of the retina of the eye (Landrum and Bone, 2001). Lutein and zeaxanthin are the primary carotenoids found in the macular region. Sufficient quantities of these nutrients in the diet are thought to reduce the risk of age-related macular degeneration, a

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Table 1. Nutritional value of one large egg.

S/N	Nutrient	Amount
1	Calories	70 kcal
2	Total fat	4.5 g
3	Saturated fat	1.5 g
4	Polyunsaturated fat	0.5 g
5	Monounsaturated fat	2.0 g
6	Cholesterol	213 mg
7	Sodium	65 mg
8	Potassium	60 mg
9	Total carbohydrate	1 g
10	Protein	6 g

Source: Hargis (1988).

leading cause of blindness in the elderly.

In addition to possibly reducing the risk of macular degeneration, lutein has been associated with a protective effect for early atherosclerosis. Dwyer et al. (2001) reported that increased amounts of dietary lutein from green leafy vegetables and egg yolks could be protective against atherosclerosis by slowing the progression of atherosclerotic lesions in humans and animals. Early arteriosclerosis was inversely related to levels of plasma lutein which were affected by dietary intake indicating an inverse relationship between dietary lutein and arteriosclerosis development.

Choline is a nutrient naturally found in eggs that has been identified as contributing to fetal memory and brain development. Choline is found in the form of phosphatidylcholine and sphingomyelin, which are types of phospholipids. Choline's chief function in the body is as an important part of cellular compounds such as the neurotransmitter acetylcholine and lecithin, a naturally occurring emulsifier present in cell membranes and bile. One large egg contains approximately 300 mg choline. Eggs are good sources of choline since the recommended daily intakes range from 425 to 550 mg for adults, including pregnant and lactating women, according to the National Academy of Sciences (Yu and Sim, 1987).

Eggs naturally contain essential and functional nutrients to promote health. In addition, the nutrient content of eggs can be modified to provide nutrients above and beyond what is normally found in generic shell eggs.

Surprisingly, even with the clear picture of the advantages of eggs and its low cost when compared to other protein sources like meat, fish and other animal proteins, the consumption of eggs per day has been found to be on the decline in recent years.

DESIGNER EGGS

Designer eggs are eggs produced when hen is fed with special feed prepared to suite the nutrients one desires to be present in the egg produced. The benefits of the designer eggs are manifold; the one of highest order is the benefit it offers in terms of the fatty acid content.

Fatty acid content

Genetic selection of hens for lowered cholesterol has not been successful in lowering the egg cholesterol content. Thus, research into lowering egg cholesterol has centered mostly on diet and pharmacological intervention (drugs). Drugs have been successful in lowering egg cholesterol by as much as 50% (Sim et al., 1973). Drugs lower cholesterol in the egg by either inhibiting the synthesis of cholesterol in the hen or by inhibiting the transfer of cholesterol from the blood to the developing yolk on the ovary. But, the drugs which have shown promise in lowering cholesterol are not yet approved by the Food and Drug Administration (FDA) for commercial use.

Research has also shown that the most effective way to lower egg cholesterol content is alter the diet of the hen. Thus, introducing the concept of designer eggs, a designer egg is and egg laid by chicken feed with a special diet of feed.

Clinical and epidemiology research has proved that the consumption of small quantities of Omega-3 fatty acids (0.5 g/day) over a long period of time decreases the coronary heart disease mortality rate (Sim, 1990).

Altering the total fat content in the diet of the hen has little effect on the total fat content of the egg yolk. However, the fatty acid profile (or the ratios of the different types of fatty acids) of egg yolk lipid can easily be changed, simply by changing the type of fat used in the diet.

Consumption of polyunsaturated fatty acids has been reported to reduce the risk of atherosclerosis and stroke. Consumption of these fatty acids has also been shown to promote infant growth. Different feeds, such as flaxseed (linseed) (Caston and Leeson, 1990; Jiang et al., 1992; Nowokolo and Sim, 1989; Sim, 1990), safflower oil, perilla oils (Shrimpton, 1987), marine algae (Hargis, 1988) fish, fish oil (Hargis et al., 1991; Yu and Sim, 1987) and vegetable oil have been added to chicken feeds to increase the Omega-3 fatty acid content in the egg yolk.

The use of Omega-3 fatty acid rich eggs, showed a reduction in plasma and liver total cholesterol produced by 20 and 40%, respectively (Sim et al., 1973).

Omega-3 fatty acid-rich eggs may provide an alternative food source for enhancing intake of these 'healthy' fatty acids. Studies confirmed that designer eggs rich in Omega-3 fatty acids though rich in cholesterol do not provoke plasma cholesterol production instead suppress the low-density lipoprotein (LDL)-cholesterol production and increase the high-density lipoprotein (HDL)-cholesterol production (Sim et al., 1973).

Studies of the eggs during storage indicated that the shelf life of the enriched eggs was comparable to that of typical eggs (Sim et al., 1973).

Many Omega-3 fatty acid-enhanced eggs are available in the U.S. market under various brand names such as Gold Circle Farms, Egg Plus, and the Country Hen Better Eggs (Sim, 1990).

Omega-3 fatty acid-enriched eggs taste and cook like any other chicken eggs available in the grocery store. However, they typically have a darker yellow yolk.

Additional benefits

Mineral content

There has been very little success in changing the calcium and phosphorus content of the albumen and yolk. It is possible, however, to increase the content of selenium, iodine and chromium (Yu and Sim, 1987). This has been done through dietary supplementation of the hen. These three minerals are important in human health.

Vitamin content

Designer eggs that have been produced contain higher concentrations of several vitamins. Two vitamins, A and E, are receiving the most interest as components of designer eggs. The vitamin content of the egg is variable and is dependent on the dietary concentration of any specific vitamin.

Pigment content

The colour of the yolk is a reflection of its pigment content. In addition, the type of pigment in the egg and its concentration are directly influenced by the dietary concentration of any particular pigment. Yolk colors can be achieved by using only natural pigments obtained from natural raw materials. Natural sources can be from plants such as marigold, chili, or corn. The high protein blue-green algae known as *Spirulina* has also been shown to be a very efficient pigment source for poultry skin and egg yolk (Landrum and Bone, 2001).

Recent research has shown that eggs may be beneficial in preventing macular degeneration; a major cause of blindness in the elderly. A recent study indicated that higher intake of carotenoids reduced the risk of age-related macular degeneration. The most effective carotenoids were lutein and zeaxanthin, which are commonly found in dark-green leafy vegetables, such as spinach and collard greens. Most of the carotenoids in egg yolk are hydroxyl compounds called xanthophylls. Lutein and zeaxanthin are two of the most common xanthophylls found in egg yolk. Lutein and zeaxanthin are high in pigmented feed ingredients such as yellow corn, alfalfa meal, corn gluten meal, dried algae meal, and marigold-petal meal (Landrum and Bone, 2001).

Fortunately, both lutein and zeaxanthin are efficiently transferred to the yolk when these various feed ingredients are fed to laying hens. With a growing problem of macular degeneration in the elderly, the egg industry may want to seize this opportunity to produce lutein and zeaxanthin rich eggs.

WHY INTRODUCE DESIGNER EGGS IN NIGERIA

Traditionally, food products have been developed for taste, appearance, value, and convenience for the consumer. Epidemiological findings, supported by animal studies, have led to recommendations that people should consume at least two servings of fruit (like apples, grapes, bananas etc.) and three servings of vegetable (carrots, green peas, cabbage, tomatoes etc.) daily. Majority of Nigerians falls well short of meeting these guidelines. The population face severe issues with malnutrition most of which goes unnoticed until complications arise and thus can benefit greatly from the consumption of eggs.

Recent reports of increase in cardiac problems among the Nigeria population have be documented, indicating an increase in cholesterol in the population this may be due to the high animal fat consumption in the country. The Nigeria populations are inclined to the consumption of animal products which consists of more than 60% of total lipids, 70% saturated fats and 100% cholesterol. A large egg contains approximately 200 to 220 mg of cholesterol (Simopolous, 1991). Thus, it is of importance to look into means of combating the resultant heart diseases and cardiac problems faced by the population. Thus it may be of interest for Nigeria to tap into the benefits offered by designer eggs.

Consumption of polyunsaturated fatty acids has been reported to reduce the risk of atherosclerosis and stroke. Consumption of these fatty acids has also been shown to promote infant growth (Simopolous, 1991). For a long time the only dietary source of Omega-3 fatty acids was from fish and studies have shown that the consumption of these fatty acids protect against cardiovascular and inflammatory diseases as well as certain kinds of cancers (Beare-Rogers, 1991; Simopolous, 1991).

Other benefits include reduction in plasma triglycerides, blood pressure, platelet aggregation, thrombosis and atherosclerosis (Simopolous, 1991).

Different feeds, such as flaxseed (linseed) (Caston and Leeson, 1990; Jiang et al., 1992; Nowokolo and Sim, 1989; Sim, 1990), safflower oil, perilla oils (Shrimpton, 1987), marine algae (Hargis, 1988) fish, fish oil (Hargis et al., 1991; Yu and Sim, 1987) and vegetable oil have been added to chicken feeds to increase the Omega-3 fatty acid content in the egg yolk.

Recent research has also shown that eggs may be beneficial in preventing macular degeneration, a major cause of blindness in the elderly. Thus, the development of eggs rich in lutein and zeaxanthin will also be of interest to the elderly population.

Thus, designer egg enriched in Vitamin E, lutein and Omega-3 fatty acids cannot be only a good nutritional product but also a good vector for the delivery of four essential nutrients vital for human health. A crucial feature of these designer eggs is the synergistic combination of healty fatty acids with major antioxidants, Vitamin E and lutein, as an important approach to the improvement of the human diet. These eggs will not be able to replace vegetable and fruits as a major source of natural antioxidants and fish products but can substantially improve the diet, especially in a country like Nigeria, significantly contributing to the recommended daily intake of essential nutrients.

Since most of the substances or raw materials required for the production of the designer feed such as yellow corn, dried algae meal, dried fish meal and alfalfa meal which can be used for the production of designer eggs are commonly found in Nigeria, its production in house can be easily carried out. But in order to implement the production of designer eggs it is important that studies be conducted using different combinations of feed in order to attain the desired nutrient values which will suite the Nigerian population requirements.

Conflict of Interests

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

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Full Length Research Paper

The effect of plant mulching and covering on the lettuce yield and nitrate content (*Lactuca sativa* L.)

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The greenhouse vegetable production is one of the most intensive in the plant production. In the greenhouse experiment established at the Economy of Agricultural Institute of Republic of Srpska, Banja Luka, during the year 2010 and 2011, the influence of mulching and plant covering on lettuce yield and nitrate content was analyzed. Lettuce vegetation period lasted 60 days. Mulching material was included in the experiment with the following variants: control (*V1*), black foil (*V2*), white foil (*V3*), black foil and agrotextile (*V4*), white foil and agrotextile (*V5)* and agrotextile (*V6*). This research has shown that mulching significantly increased the lettuce yield, accordingly variants with mulching ranged from 70.91 t ha^{-1} (V5) to 104.14 t ha^{-1} (*V2*), in average of two-year research. The highest average nitrate content in two-years research was registered in the control treatment (*V1*), and the lowest in the soil mulching with black foil and covering plants agrotextile (*V4*).

Key words: Greenhouse, agrotextile, mulching, nitrates, lettuce.

INTRODUCTION

Lettuce is one of the most commonly grown vegetable crops in greenhouses, that is characterized by the large amounts of nitrates accumulation in fresh green mass. Nitrates that due in the human organism are mainly derived from vegetables 70% (especially leafy), about 20% from drinking water if it contains up to 10% mg NO_3^- (Kastori and Petrovic, 2003). Agrotextil increases yield and reduction of nitrate in lettuce (Tosic et al., 2013). Since the lettuce is used for the human consumption in fresh condition, a high concentration of nitrates can cause health problems (e.g. methemoglobinemia). This problem is particularly important in the case of leafy vegetables such as lettuce, which are capable of high NO_3^- accumulation (Elkner and Kaniszewski, 2001; Premuzic et al., 2002). For this reason the nitrate content in fresh vegetables is regulated by the law in certain countries. Concerns about high nitrate concentrations in vegetables have prompted the European Union to introduce limits on

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Table 1. Scheme experiment.

*V_4	0.75	*V_5	0.75	*V_6	0.75	*V_1	0.75	*V_2	0.75	*V_3
1		1		1		1		1		1
*V_5	0.75	*V_6	0.75	*V_1	0.75	*V_2	0.75	*V_3	0.75	*V_4
1		1		1		1		1		1
*V_6	0.75	*V_1	0.75	*V_2	0.75	*V_3	0.75	*V_4	0.75	*V_5
1		1		1		1		1		1
*V_1	0.75	*V_2	0.75	*V_3	0.75	*V_4	0.75	*V_5	0.75	*V_6

V: variants, V1 control, V2 black foil, V3 white foil, V4 black foil+agrotextil, V5 white foil+agrotextil, V6 agrotextil, ***plants (68 per variants.

the nitrate concentration in some salad crops, including lettuce, in order to reduce nitrate intake by consumers.

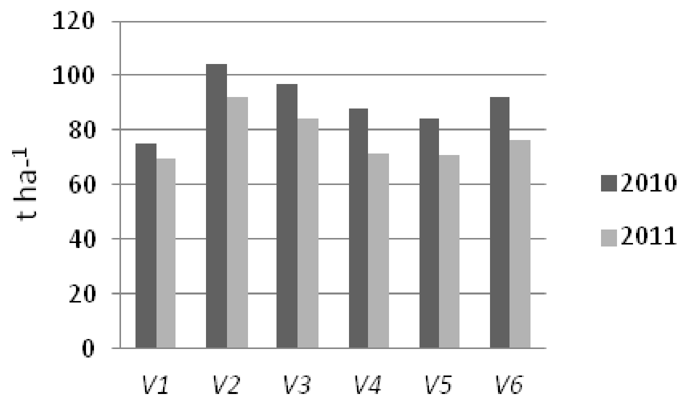
MATERIALS AND METHODS

In order to study the impact of the mulching and agrotextile plant coverage on lettuce yield and nitrate content the experiment is set on the Economy of Agricultural Institute during the period from March to May 2010 and from March to May 2011, at the greenhouse without heating, on the alluvial soil. The experiment was set by a randomized block system with six replications in four variants, with the size of the basic plot of 5 m^2. Sowing was done in salad containers, intended for seedling production. Planting was done manually, and a lettuce variety "Nizzi" was used. Planting seedlings of lettuce was 6th March. Harvesting lettuce is done at the stage of technological maturity, 6th May. For ground covering used a black foil (width 1.2 and 5.5 m thick) and white film (width 1.2 m and length 5.5 m), and immediately cover the plants agrotextil. Soil mulching with black and white film, as well as cover plants agrotextile was performed 5th March. Lettuce planting was conducted in the early March, with the inter-row spacing of 25 cm and the spacing in a row of 30 cm (13.6 plants per m^2, that is,. 68 plants on 5 m^2). Lettuce vegetation lasted for sixty days. The black and white PE foil was used for mulching, and agrotextile for point-blank plant covering, with the following variants: control (V_1), black PE foil (V_2), white PE foil (V_3), black PE foil and agrotextile (V_4), white PE foil and agrotextile (V_5), and agrotextile (V_6). Black and white foil was used for ground covering, and agrotextile for point-blank plant covering (Table 1).

Lettuce harvesting was made in the stage of technological maturity. During the growing season the soil moisture was maintained at an optimum level, by the system of drop by drop. After the extraction with distilled water the lettuce nitrate content was determined by spectrophotometry (Johnson and Ulrich, 1950). Throughout the harvest, in order to determine the total lettuce yield, the weight of 20 plants was measured from each repeated variation, while for the determination of nitrate content the four lettuce plants were used. The statistical significance of differences was tested by LSD test.

RESULTS

The average lettuce yield is shown in Table 1, where highest yields in the greenhouse lettuce production, in two-years of research, were achieved by soil mulching



Graph 1. The average lettuce yield (t ha^{-1}).V: variant, V1 control, V2 black foil, V3 white foil, V4 black foil+agrotextil, V5 white foil+agrotextil, V6 agrotexti.

with black (V_2) and white (V_3) PE foil (Graph 1). The soil mulching application had a significant effect on lettuce yield in the both years of research. Lettuce yield in the mulching variants is significantly higher than in the control variants. Table 2 shows the average nitrate content in lettuce fresh mass, where in the first year of research the lowest nitrate content was registered in soil mulching with black PE foil and agrotextile plant covering, and the highest in soil mulching with black PE foil. In the second year of study the highest nitrate content is registered in control variant and the lowest in soil mulching with black PE foil (Graph 2 and 3).

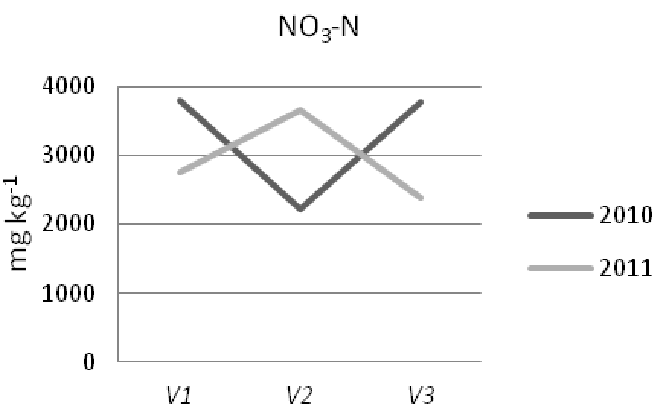
DISCUSSION

Compared to the control, in the two-years of research, all variants achieved a higher average yields per plant. Better results were achieved in mulching variants, without agrotextile plant covering. Mulching has a positive effect on plant growth, increase of vegetative mass and yield (Maged, 2006). The difference between these variates

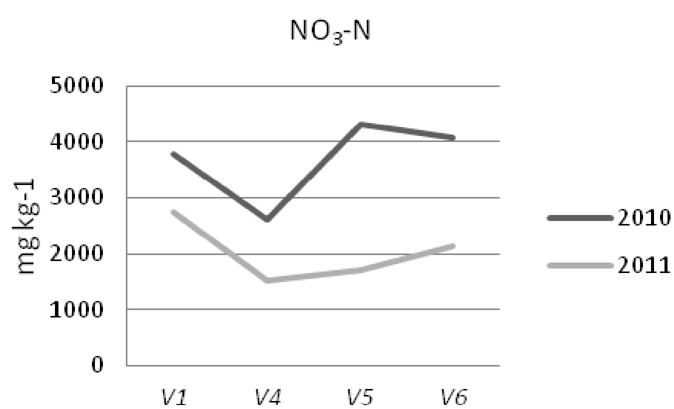
Table 2. The average lettuce yield (g plant^{-1}).

Variants		Average yield 2010 (g biljci^{-1})	Average yield 2011 (g biljci^{-1})	Average yield 2010 and 2011(g biljci^{-1})
V1		553.25	512.87	533.06
V2		765.77	676.53	721.15
V3		713.68	620.49	667.09
V4		648.34	529.16	588.75
V5		617.65	521.40	569.53
V6		678.13	560.41	619.27
Average		662.80	570.14	616.47
F-test		147.1	84.8	
LSD	0.05	18.49	14.22	
	0.01	25.57	19.66	

V: variants, V1 control, V2 black foil, V3 white foil, V4 black foil+agrotextil, V5 white foil+agrotextil, V6 agrotextil, * statistically significant difference, ** statistically highly significant difference.



Graph 2. The average nitrate content in lettuce. V: variants, V_1 control, V_2 black foil, V_3 white foil.



Graph 3. The average nitrate content in lettuce. V: variants, V_1 control, V_4 black foil+agrotextil, V_5 white foil+agrotextil, V_6 agrotextil.

and control is significant. Growing plants in not mulching soil proved to be the worst options for realization total yield. The highest yields in the greenhouse lettuce production, in the both years of research, were achieved in soil mulching with black and white PE foil. Plant growing on the uncovered soil proved to be a bad solution for the total yield achievement. Increase of the yield for variants with white and black PE foil was registered. Similar results came Balalić (2004) who found that the weight of lettuce was 54% higher in the treatment with the combined application of PE foil and agrotextil (358 g) compared with not mulching variant (232 g). In autumn production the largest plant mass was registered in a high tunnel, using the white and black mulch foil, that amounted to 288.28 g plant^{-1} (Ponjičan and Bajkin, 2004). Plants grown in different variants of mulching (PE foil, agrotextil, agrotextil PE foil and agrotextil) achieved significant or highly significant differences in the studied parameters compared to plants grown in bare soil. These results are confirmed by many authors (Nguyen, 2006; Đurovka and Ilin, 2002). The results obtained in our experiment are consistent with a numerous research by showing that the yield of lettuce depends primarily on the variety, the conditions of production, nutrition, planting dates and harvest (Conversa et al., 2004). During the two years of research (Siwek et al., 2007) found that the black mulch has a positive effect on the vegetables yield in relation to the white and transparent foil, which is according with the results of this study. Studies on the effect of different material types by Deiser and Eichin (1992), and their effect on lettuce yield, shows that the darker materials have a greater effect on yield than the lighter materials due to the creation of more favorable soil conditions, especially better utilization of nitrogen. Plant covering influences the maturity and yield (Ponjičan and Bajkin, 2004).

In the 2011 significantly lower lettuce nitrate content was recorded compared to the 2010. Average nitrate content in lettuce in mulching variants of two-year research, ranged from 2065,5 mg NO_3^- kg^{-1} (*V4*) to 3266,5 mg NO_3^- kg^{-1} (*V1*). Minimal average nitrate content in lettuce in 2010 was registered in *V2* (2227 mg NO_3^- kg^{-1}), and maximal average nitrate content in lettuce was registered in *V5* (4308 mg NO_3^- kg^{-1}). The highest content of nitrate in 2011 is registered in *V2* (3660 mg NO_3^- kg^{-1}), and the lowest in *V4* (1520 mg NO_3^- kg^{-1}). The obtained values are in line with the announcement (Balalić, 2004) that the conditions of the protected area in the variant combination of mulching+agrotextil, stated value of 2167 mg kg^{-1} of nitrates in lettuce. During the two-year research (Wojciechowska et al., 2007) concluded that soil mulching has a significant impact on the reduction of nitrate content in lettuce heads compared to the control treatment, which corresponds with results of this research. (Escobar-Gutierrez et al., 2002) found that several long-day lettuce varieties had exceeded the maximum nitrate permissible limit of 3500 mg NO_3^- kg^{-1}, while the relatively small number of short-day varieties had a higher nitrate content in the winter sowing than it is allowed 4500 mg NO_3^- kg^{-1}. In studies (Govedarica-Lučić and Perkovic, 2013) found that the average value of nitrate ranged from 2197.25 mg/kg (control) to 2526.25 mg kg^{-1} (agrotextil). In the 2010 during the last week of vegetation plants were exposed to the different conditions, especially high temperatures, that can also affect the nitrate content in fresh mass.

Conclusion

Based on the two-year research it can be concluded that the soil mulching had a positive effect on yield increase compared to the control variant. Better results were achieved in mulching variants, without agrotextile plant covering. Agrotextil had a positive effect on the reduction of nitrate in lettuce. In the first year of research during the last week of vegetation plants were exposed to different conditions, especially high temperatures, which influenced the increase of nitrates in lettuce fresh mass. The results of these studies show that in the variation of soil mulching with black foil along with agrotextile plant covering in both years of research, although conditions in the first year were not favorable, and that the lowest lettuce nitrate content was registered.

Conflict of Interests

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

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Full Length Research Paper

Composted municipal solid waste and NPK fertilizer effect on yield, yield components and proximate composition of maize

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Two year experiment was conducted in the Teaching and Research Farm of Department of Crop Science, Faculty of Agriculture, University of Nigeria, Nsukka to: compare the effect of combined municipal solid waste (MSW) and NPK fertilizer application on yield components, yield and proximate composition of maize. A 4 × 4 factorial experiment in Randomized Complete Block Design (RCBD) with three replications was used for the study. The two factors: MSW (0, 1000, 1500 and 2000 kg/ha) and NPK (20:10:10) (0, 100, 200 and 300 kg/ha) were combined to get 16 treatment combinations. Sole application of 2000 kg/ha of MSW and 300 kg/ha of NPK rates were observed to achieve the highest yield of maize in 2011 and 2012 seasons respectively among their treatment rates. Complementary application of MSW with NPK was significantly higher than either sole application with respect to plant attributes measured. Combination of 2000 kg/ha of MSW with 300 kg/ha of NPK had significantly higher effect than other interactions in both years and was found satisfactory to achieve the best yield performance and proximate composition of maize in the study.

Key words: Municipal solid waste, NPK, maize yield, yield components, proximate composition.

INTRODUCTION

municipalities are facing a growing problem of how to safely dispose off their solid waste. Bryan and Morton (2007) define municipal solid waste (MSW) as waste from multifamily, commercial, and institutional e.g. schools, government offices. This definition excludes many materials that are frequently disposed with MSW in landfills including combustion ash; water waste treatment residuals, construction and demolition waste, and non hazardous industrial process waste (U.S EPA, 2007).

In most developing countries like Nigeria, MSW are used as landfills or dumped in sites where people no longer use. In some sites where MSW are dumped, they are being burnt after heap of it is large and overflows the site at which it is dumped. Many strategies are being adopted to dispose wastes but most of them are neither safe to the environment nor sustainable for nutrient

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conservation (Rizwan et al., 2006). Municipal solid wastes compose of prutesible (biodegradable) and non prutesible (non-biodegradable) constituent. Prutesible fraction includes waste from kitchen, paper industries, wood scraps and others. Non prutesible includes waste from rubber, automobile and polythene industries and others.

Oyinlola (2001) reported that the organic components account for about 76% of total MSW in Nigeria. Anton et al. (2005) stressed that in order to minimize environmental impacts and the loss of organic resources, there should be measures taken to increase and improve sorting at origin, recuperation and recycling, including compositing of organic and green MSW. Organic MSW is described as household waste and other waste which because of its nature of composition, is similar to household waste, capable of undergoing anaerobic or aerobic decomposition, excluding green MSW from gardens and parks, which includes tree cuttings, branches, grass and wood (European Commission, 2001). Composting has been one of the best solutions to reduce the huge pile of organic waste and its conversion to a value added product. It is one of the major recycling processes by which nutrients present in organic materials are returned back to the soil in plant available form (Inckel et al., 1996). The application of organic manure to soil provides benefits including improving the fertility, structure, water holding capacity of soil, organic matter in the soil and reducing the amount of synthetic fertilizer needed for crop production (Phan et al., 2002; Blay et al., 2002).

The low fertility status of most tropical soils hinders crop production as most crops have a strong exhausting effect on the soil. It is generally observed that crops fail to produce reasonably in plots without adequate nutrients. Inorganic fertilizer such as NPK exerts strong influence on plant growth, development and yield (Stefano et al., 2004). The availability of sufficient growth nutrients from inorganic fertilizer lead to improved cell activities, enhanced cell multiplication and enlargement and luxuriant growth (Fashina et al., 2002). However excessive or continual use of NPK will lead to loss of soil fertility due to improper use of it which has adverse impact on agricultural productivity, caused soil degradation and even contaminate underground water resources.

Integrated use of organic nutrient sources with inorganic fertilizer was shown to increase the potential of organic fertilizer (Heluf, 2002) and improve the efficiency of inorganic fertilizer. Incorporation of chemical fertilizers in composted materials improves its efficiency and reduces losses (Gua and Geta, 1993). Rizwan et al. (2006) reported that, the integrated use of organic and inorganic plant nutrient sources not only reduces organic waste causing environmental pollution but also conserves rich pool of nutrients resources, which can reduce the sole dependence on chemical fertilizer. High and sustained crop yield can be obtained with judicious balance of NPK fertilization combined with organic matter amendment.

Maize has immense potential in the tropics and yields up to 7.5 t/ha of grain if the crop is properly managed (Kolawole and Joyce, 2009). Unfortunately, yields are still generally below 5 t/ha in Nigeria (FAO, 2007), and this has caused inadequacy of maize for its numerous usages. Yield differences within temperate areas have been attributed to low nutrient status of its soils especially nitrogen, phosphorus and potassium resulting from the practice of slash and burn farming system associated with bush fallow and excessive leaching of the soil nutrients. This system (slash and burn) is presently unsustainable due to high population pressure and other human activities which have resulted in reduced fallow period (Steiner, 1991). This study was conducted to achieve the following objectives: to compare the response of maize to combined MSW compost and NPK fertilizer application on yield, yield components and to determine the effect of MSW compost and NPK fertilizer on the nutrient composition of maize.

MATERIALS AND METHODS

Two year field experiment was carried out at the Department of Crop Science Teaching and Research Farm, University of Nigeria, Nsukka which is located at latitude 06° 521 N and longitude 07° 241 E with a 447 m altitude above sea level. It is within the lowland humid tropical agro ecology of Nigeria. The municipal solid waste used for the study was generated from waste bins found within University of Nigeria, Nsukka campus. The biodegradable materials were properly sorted before the materials were composted aerobically. The composted material included: food waste, paper, vegetable scrap, plant leaves, banana, cassava and pawpaw peelings and plant cuttings. The composted product used in the study was free of odour and dark in colour, but with small amount of foreign materials as defined by CCME (2000). The chemical fertilizer used was NPK (20:10:10) which was sourced from ENADEP depot in Nsukka. Figures 1, 2, 3 and 4 show stages of compost maturity during composting.

The experimental design was a 4 × 4 factorial in randomized complete block design (RCBD). The two factors: MSW compost (0, 1000, 1500 and 2000 kg/ha) and NPK 20:10:10 (0, 100, 200 and 300 kg/ha) were combined to produce 16 treatment combinations (Table 1) which was replicated three times. Two weeks after planting (WAP), the treatments were applied to the plots accordingly. The treatments were applied in a ring form (10 to 15 cm from the plant) and properly mixed with the soil at the base of the plant. The following parameters were measured: fresh weight, dry weight, ear length, ear weight, ear circumference, cob weight, 100 grain weight, shelling percentage, harvest index and grain yield. The harvested grains from the plots were analyzed in the laboratory to obtain their proximate composition.

Harvest index: This was obtained by calculating from the formula:

$$\frac{\text{Seed weight } (g/\text{plant})}{\text{Total dry matter of plant material} (g/\text{plant})}$$

Data was subjected to analysis of variance (ANOVA); means were separated using Fisher's least significant difference (F- LSD) procedure as described by Obi (2002). Test of significance was at 5% probability level.

Figure 1. Raw material used for the compost.

Figure 2. First stage of maturity.

Figure 3. Another stage of maturity.

Figure 4. Final stage (Matured compost) ready for use.

RESULTS AND DISCUSSION

Table 2 shows the soil physicochemical properties. The soil is sandy loam in texture and moderately acidic. It is low in organic matter content, total nitrogen and exchangeable bases but had relatively moderate cation exchange capacity (CEC) and available phosphorous values. The low fertility status of the soil is common to most tropical soils due to their advance stage of weathering and high leaching tendency (Ibedu et al., 1988).

The chemical analysis of the municipal solid waste (MSW) showed that the MSW had organic matter (18.3%), total nitrogen (1.4%), potassium (0.28%) and available phosphorous (0.05%) but had a high pH value (8.3) which is capable of ameliorating the acidic content of the soil (Table 3). The MSW pH used for the study was high in water when compared the pH values of the experimental site. This suggests that application of the compost could have buffered the soil which helped the soil to provide the needed nutrient required by the plant after the compost was applied (Onwudiwe et al., 2013). Application of MSW could have also improved the soil structure, porosity, aeration, drainage and moisture holding capacity according to Bresson et al. (2001).

Application of 300 kg/ha NPK had the highest fresh weight of plant among NPK rates. Also, 2000 kg/ha MSW produced the highest fresh weight of plant among MSW rates. It was observed that combination of 2000 kg/ha of MSW with 300 kg/ha of NPK produced the highest fresh weight of plant in both years. The result also showed that application of 2000 kg/ha of MSW with 300 kg/ha of NPK gave the highest dry weight of plant among the treatment interactions.

Ear length was observed to perform highest with the application of 2000 kg/ha of MSW while 300 kg/ha of NPK also acted same among treatment rates in both years. Combined rates of 2000 kg/ha of MSW with 300 kg/ha of NPK produced the highest on ear length in both years. Application of 2000 kg/ha MSW with 300 kg/ha NPK in year 2012 produced the highest cob weight and 100 grain weight among the MSW x NPK treatment combination. Harvest index was observed to follow the same trend with cob weight and 100 grain weight (Table 4). Application of 2000 kg/ha with 300 kg/ha of NPK was observed to perform significantly higher ($P < 0.05$) than other combinations on shelling percentage in 2011 while 2000 kg/ha of MSW with 200 kg/ha of NPK produced the

Table 1. Treatment combinations used for the study.

NPK fertilizer (kg/ha)	MSW kg/ha			
	0	1000	1500	2000
0	0/0	1000/0	1500/0	2000/0
100	0/100	1000/100	1500/100	2000/100
200	0/200	1000/200	1500/200	2000/200
300	0/300	1000/300	1500/300	2000/300

Table 2. Physical and chemical properties of the experimental site prior to planting.

Physical properties	Particle size
Coarse sand (%)	40
Fine sand (%)	31
Clay (%)	24
Silt (%)	24
Class	Sandy clay loam
Chemical properties	Value
P^H in water	4.7
P^H in KCl	3.8
Organic carbon (%)	0.92
Organic matter	1.58
Total N (%)	0.028
P (ppm)	22.38
K (me/100 g)	0.25
Mg (me/100 g)	1.20
Ca (me/100 g)	1.40
Na (me/100 g)	0.23
CEC (me/100 g)	6.00
Exchange acidity	
Al (me/100 g)	2.00
H (me/100 g)	0.8

Table 3. Chemical characteristics of composted MSW used for the study.

Chemical properties	Value
pH in water	8.3
pH in KCl	7.8
Organic carbon (%)	10.64
Organic matter (%)	18.34
Total N (%)	1.401
P (%)	0.048
K (%)	0.279
Mg (%)	2.10
Ca (%)	4.00
Na (%)	0.558

highest shelling percentage in 2012 season. There were significant ($P < 0.05$) differences among the interaction effects in these yield components (Table 4). On grain yield per hectare, combined rate of 2000 kg/ha of MSW

Table 4. Effect MSW x NPK fertilizer rates on yield components and yield traits of maize in two years.

Year	MSW rate (kg/ha)	NPK rate (kg/ha)	FW (g)	DW (g)	EH	EW (g)	EC (cm)	COBW (g)	100GW (g)	SY/ha (kg)	H.I	SH%
2011	0	0	673.5	238.2	24.66	200.00	16.77	121.34	15.05	3477	0.27	54.02
		100	701.3	246.5	25.63	216.67	17.02	127.31	15.37	3764	0.28	55.77
		200	728.4	249.9	25.90	225.00	17.21	129.55	15.69	4111	0.30	59.86
		300	756.7	257.0	26.20	237.33	17.33	132.93	15.95	4299	0.31	61.04
	1000	0	763.5	254.8	25.83	238.33	17.25	129.88	15.85	4213	0.30	61.24
		100	803.3	265.3	26.64	242.00	17.51	138.07	16.26	4652	0.32	64.05
		200	823.7	268.6	26.90	246.67	17.76	140.56	16.36	5029	0.34	67.74
		300	857.7	275.9	27.18	250.67	17.87	142.39	16.58	5354	0.35	71.31
	1500	0	839.0	271.9	26.95	246.67	17.75	139.86	16.37	5041	0.34	68.33
		100	883.2	282.8	27.60	257.33	18.44	147.08	16.81	5352	0.35	69.14
		200	911.9	288.4	27.63	262.50	18.65	150.59	17.10	5818	0.38	73.34
		300	942.3	294.1	28.46	266.67	18.80	154.44	17.48	5978	0.38	73.56
	2000	0	954.3	293.4	28.16	259.33	18.94	153.37	16.92	5865	0.37	72.76
		100	984.1	300.0	28.66	271.67	19.26	157.26	18.97	6364	0.39	76.74
		200	1011.7	310.0	28.97	277.00	19.45	162.99	19.54	6455	0.38	75.29
		300	1037.8	315.3	29.25	286.00	20.30	165.84	19.76	6822	0.40	78.09
2012	0	0	667.5	272.0	25.37	189.20	16.18	159.60	36.00	2978	0.18	35.88
		100	817.5	556.7	26.37	229.00	14.52	185.30	22.00	3228	0.18	33.89
		200	891.6	654.0	27.21	229.90	17.36	193.90	24.00	3605	0.18	36.70
		300	846.7	700.0	27.40	234.70	17.88	194.60	27.00	3681	0.19	34.40
	1000	0	920.5	641.7	26.96	240.80	17.63	215.00	25.00	3818	0.18	37.25
		100	979.5	687.0	27.75	248.80	16.48	216.90	27.00	3994	0.20	35.19
		200	1020.0	708.7	29.09	264.80	16.97	223.90	25.00	4127	0.21	35.09
		300	1058.7	715.3	30.01	274.20	18.45	228.90	28.00	4358	0.23	36.20
	1500	0	1018.2	692.7	28.00	256.60	17.63	206.30	26.00	3066	0.22	40.59
		100	1093.7	730.0	27.88	264.10	16.88	231.70	28.00	4584	0.30	37.59
		200	1118.0	737.3	28.54	281.00	17.23	255.00	29.00	4766	0.25	38.67
		300	1140.0	752.0	29.10	283.20	17.82	237.40	30.00	4996	0.26	40.45
	2000	0	1068.3	725.0	28.63	271.10	17.41	228.94	27.00	5153	0.26	41.56
		100	1135.0	765.2	27.60	286.40	17.09	238.37	30.00	5258	0.27	41.92
		200	1152.3	784.0	28.95	299.20	17.41	238.00	30.00	5603	0.28	44.70
		300	1186.0	805.7	29.90	283.70	18.16	258.00	31.67	5828	0.29	43.06
		$F\text{-}LSD_{0.05}$	64.44	59.65	1.40	10.70	1.17	6.22	9.20	526.5	0.05	9.37

Where FW= Fresh weight, DW= dry weight, EL= ear weight, EC=ear circumference, CobW= cob weight, 100GW= 100 grain weight, SY/ha= seed yield per hectare, H.I= harvest index, SH%= shelling percentage.

with 300 kg/ha of NPK resulted to the highest yield among the treatment combinations.

Irrespective of weather condition of the planting seasons, the increase in MSW resulted to increase in yield components and yield. Municipal solid waste rate of 2000 kg/ha achieved the best yield traits among MSW rates in both planting seasons. This suggested that the increase in rate of MSW resulted to the increase in grain

Table 5. Effect of year on yield components and yield traits of maize plant in 2011 and 2012 planting seasons.

Year	Traits									
	FW 9g)	DW (g)	EL (cm)	EW (g)	EC (cm)	COBW (g)	100GW (g)	SY/ha (kg)	H.I	SH%
2011	854.5	275.8	27.16	248.96	18.14	143.35	16.86	5162	0.34	67.64
2012	1007.1	683.0	28.05	258.11	17.19	218.24	28.04	4315	0.23	38.32
$F\text{-}LSD_{0.05}$	16.11	14.91	0.35	2.67	0.29	1.55	2.30	131.6	0.01	2.24

Where FW= Fresh weight, DW= Dry weight, EL= Ear length, EW= Ear weight EG=Ear circumference, COBW= Cob weight, 100GW= 100 Grain weight, SY/ha= Grain yield per hectare, H.I= Harvest index, SH%= Shelling percentage.

Table 6. Meteorological data of the experimental site in 2011 and 2012.

Month	2011						2012					
	Rainfall (mm)		Temperature (°C)		Relative humidity (%)		Rainfall (mm)		Temperature (°C)		Relative humidity (%)	
	Total (mm)	Days	Max	Min	10AM	4PM	Total (mm)	Days	Max	Min	10AM	4PM
Jan.	0.0	0	32.1	18.4	57.1	44.7	0.0	0	31.7	19.8	58.2	48.7
Feb.	54.9	3	32.3	22.1	73.8	60.5	23.1	3	31.8	21.7	73.6	61.3
Mar.	14.5	2	33.5	23.0	72.2	57.7	0.0	0	33.4	23.0	71.3	53.4
Apr.	87.1	8	30.8	22.0	74.3	65.1	103.9	4	31.4	22.4	73.8	62.8
May	140.5	12	31.0	21.9	74.5	70.1	282.1	13	30.2	21.0	74.1	67.8
June	127.3	12	29.9	21.5	75.7	71.3	193.6	13	28.4	20.3	75.8	71.5
July	192.3	13	28.0	21.0	75.7	72.5	276.1	20	27.8	20.3	75.4	72.3
August	149.1	14	27.0	20.7	76.5	74.0	0.0	0	26.6	20.1	74.6	71.5
Sept.	254.0	15	28.0	20.7	76.8	74.0	307.5	16	27.7	20.4	75.8	75.3
Oct.	184.0	12	28.3	20.9	75.6	72.1	291.6	18	28.3	20.1	73.2	73.0
Nov.	28.0	2	30.3	20.8	69.3	59.5	61.0	4	30.1	21.6	73.8	73.8
Dec.	0.00	0	31.6	16.7	56.5	47.4	0.0	0	30.9	18.7	75.0	75.0
Total	**1231.7**	**93**	**362.8**	**228.9**	**818.2**	**768.9**	**1538.9**	**91**	**358.3**	**227**	**950.4**	**806**

filling and dry matter content of plant which resulted to appreciable grain yield. Application of NPK rate of 300 kg/ha followed same trend as MSW. Obidiebube et al. (2012) observed same when NPK at different levels was applied on maize. Combination of 300 kg/ha of NPK and 2000 kg/ha of MSW performed better than other treatment combinations with respect to plant parameters studied in the two planting seasons. Combined application of organic and inorganic fertilizers has been reported to increase plant growth and yield (Mahmoud, 2009; Patil, 2010; Nyangani, 2010; Milosevic and Milosevic, 2009). It was also reported that incorporation of chemical fertilizers in composted materials improves its efficiency and reduces losses (Gua and Geta, 1993).

The yield components and yield were also significantly affected by year of planting. The 2012 planting season performed significantly ($P<0.05$) higher than 2011 planting season in all yield traits except in ear circumference, grain yield per hectare and harvest index that were higher in 2011 (Table 5). This could be attributed to the distribution of rainfall in the two years (Table 6). The month of august marked the silking stage of the plant, when comparing the month of august in both years it was found that there was total drought in year 2012 which explains the better yield had in 2011 (Table 6). Drought stress generally lowers yield potential either by restricting growth during the vegetative period of development and hence the subsequent capacity for photosynthesis during grain filling or by damaging the embryo ears so that the sink for photosynthesis product is reduced (Aderi, 1993). In turn, rainfall plays a major role on nutrient solubility and absorption by plant and on the translocation of photosynthates from the source to the sink (Aderi, 1993). Generally, amount of rainfall that is well distributed is needed for high maize yield.

Table 7 showed that application of NPK, MSW and their interaction had significant ($P < 0.05$) effect on moisture content of grain. The highest moisture content was obtained when 300 kg/ha rate of NPK was applied and significantly ($P < 0.05$) differed from other rates of NPK. Application of 2000 kg/ha MSW also gave the highest moisture content and differed significantly ($P < 0.05$) from other MSW rates. The combination of 2000 kg/ha of MSW with 300 kg/ha of NPK had the highest moisture content among other combinations. Increase in concentration of treatment resulted to increase in the

Table 7. Effect of composted municipal solid waste (MSW) and NPK fertilizer on proximate composition of maize.

NPK Fertilizer (kg/ha)	MSW (kg/ha)				Mean
	0	1000	1500	2000	
Moisture content %					
0	5.590	6.027	6.363	6.540	6.130
100	5.727	6.143	6.373	6.737	6.245
200	5.883	6.210	6.527	6.913	6.383
300	6.033	6.313	6.607	7.060	6.503
Mean	5.808	6.173	6.467	6.812	6.315
Ash content %					
0	1.480	2.093	2.320	2.443	2.084
100	1.700	2.127	2.377	2.447	2.163
200	1.933	2.130	2.380	2.510	2.238
300	1.933	2.313	2.393	2.527	2.292
Mean	1.762	2.166	2.368	2.482	2.194
Crude fibre %					
0	2.007	2.337	2.517	2.720	2.395
100	2.110	2.427	2.587	2.803	2.482
200	2.240	2.480	2.630	2.903	2.563
300	2.350	2.508	2.703	2.977	2.634
Mean	2.177	2.438	2.609	2.851	2.519
Crude protein %					
0	7.520	7.833	8.260	8.397	8.003
100	7.563	8.123	8.383	8.047	8.029
200	7.733	8.203	8.453	9.083	8.368
300	7.937	8.270	8.570	9.220	8.499
Mean	7.688	8.108	8.417	8.687	8.225
Crude fat %					
0	2.467	2.753	3.363	4.210	3.198
100	2.520	3.117	3.940	4.400	3.494
200	2.580	3.273	3.920	4.490	3.566
300	2.867	3.353	4.203	4.593	3.754
Mean	2.608	3.124	3.857	4.423	3.503
Carbohydrate %					
0	82.417	81.050	79.563	78.127	80.289
100	82.247	80.187	78.517	77.347	79.574
200	81.583	79.833	78.580	76.877	79.218
300	80.813	79.557	77.917	76.383	78.667
Mean	81.765	80.157	78.644	77.183	79.437

	MC	Ash C	C. fib	C.P	C.F	Cab
F-LSD$_{0.05}$ for comparing 2 NPK rates:	0.079	0.086	0.070	0.3751	0.1759	0.3414
F-LSD$_{0.05}$ for comparing 2 MSW rates:	0.079	0.086	0.070	0.3751	0.1759	0.3414
F-LSD$_{0.05}$ for comparing 2 NPK x MSW rates:	0.159	0.173	0.140	0.7502	0.1759	0.6828

Where: MC= moisture content, Ash C= ash content, C.fib= crude fibre, C.P= crude protein, C.F= crude fibre, Cab= carbohydrate.

moisture content of grains.

Application of NPK, MSW and their interaction significantly ($P < 0.05$) influenced ash content of grain (Table 7). Application of NPK at 200 and 300 kg/ha gave statistically ($P < 0.05$) similar effect but significantly ($P < 0.05$) higher than 0 kg/ha rate of NPK. Application of 300 kg/ha NPK had significantly (P < 0.05) higher ash content than 0 kg/ha of NPK. The 2000 kg/ha of MSW gave the highest ash content and differed significantly (P < 0.05) from other MSW treatments. Combination of 2000 kg/ha MSW with 300 kg/ha NPK had significantly (P < 0.05) higher ash content over 0 kg/ha of NPK with 0 kg/ha of MSW. Ash content of grain increased with increase in treatment rates.

Crude fibre was significantly (P < 0.05) influenced by the application of NPK, MSW and their interaction (Table 7). The 300 kg/ha rate of NPK gave the highest crude fibre content among NPK rates while 2000 kg/ha of MSW had the highest crude fibre among the MSW rates. Interaction of 2000 kg/ha of MSW with 300 kg/ha of NPK gave significantly (P < 0.05) higher crude fibre content than combination of 0 kg/ha of NPK with 0 kg/ha of MSW.

The application of NPK, MSW with their interaction showed significant (P < 0.05) effect on crude protein content (Table 7). The effect of 200 and 300 kg/ha rates of NPK were statistically (P < 0.05) similar but significantly (P < 0.05) higher than 0 kg/ha of NPK. The 300 kg/ha of NPK produced significantly (P < 0.05) higher crude protein than 0 kg/ha of NPK. Application 1500 and 2000 kg/ha of MSW produced statistically (P < 0.05) the same effect but significantly (P < 0.05) higher than 0 kg/ha of MSW. Interaction of 2000 kg/ha MSW with NPK 300 kg/ha obtained significantly (P < 0.05) higher crude protein than interaction of 0 kg/ha of NPK with 0 kg/ha of MSW. Increase in treatment concentration resulted to increase in grain crude protein. The finding suggests that N assimilation increased with increase in treatment application rate especially as it determined the crude protein content of the grain. Also, Table 7 showed that NPK, MSW and their interaction had significant (P < 0.05) effect on crude fat content. The application of 100 and 200 kg/ha rates of NPK were statistically (P < 0.05) the same but significantly (P < 0.05) higher than 0 kg/ha of NPK with respect to crude fat. The 300 kg/ha rate of NPK produced the highest crude fat among NPK treatments while 2000 kg/ha of MSW gave the highest crude fat among MSW rates. Combination of 2000 kg/ha of MSW with 100 kg/ha of NPK gave significantly (P < 0.05) higher crude fat over 0 kg/ha of NPK with 0 kg/ha of MSW.

The application of NPK, MSW and their interaction produced significant (P < 0.05) effect on carbohydrate content (Table 7). Zero kg/ha rate of NPK had the highest carbohydrate among other NPK rates while 0 kg/ha rate of MSW obtained the highest carbohydrate among MSW treatments. Combination of 0 kg/ha of NPK with 0 kg/ha of MSW had the highest carbohydrate content among the treatment combinations. Carbohydrate decreased with increase in concentration of treatment rates. Municipal solid waste increased proximate composition of maize grain as the application rate increased except for carbohydrate that decreased with increase in application rate. Application of NPK followed same trend with MSW. The relationship between N fertilizer and consumption of soluble carbohydrates in plants may be due to metabolism nitrogen fixation. Since some of the intermediate metabolic in TCA cycle is used for amino-acid and protein synthesis, so the amount of carbohydrate is reduced especially in crops like maize (Almodares et al., 2009). This was also observed by Savotskii and Salmanov (1972) and Ghaly (1976) that increasing N fertilizer rate of corn plant decreased the total soluble sugar % and carbohydrate % in grains of corn. Complementary application performed better than sole application. This result is in agreement with those of Akintoye and Olaniyan (2004) when they observed that fertilizer application significantly increased the values of proximate composition of maize grain. Increase in yield attributes and nutritional value of crops arising from the use of combination of organic and chemical fertilizers has also been reported (Bagheri et al., 2011). Ogbonna et al. (2012) also reported that improved nutritional value of maize grain resulting from the application of the formulations may be attributed to the increased soil fertility.

Conclusion

The experiment result showed that combined application of MSW with NPK performed better than sole application of MSW or NPK fertilizer. Municipal solid waste rate of 2000 kg/ha performed better than other lower rate while 300 kg/ha of NPK performed better than other lower rates. Combination of 2000 kg/ha of MSW and 300 kg/ha performed better than other treatment combinations and was found satisfactory to improve yields and proximate composition of maize in the study. It is recommended that further research should be carried out to determine the optimum rate required for maize production since performance increased with increase in rate of application.

Conflict of Interests

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

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Full Length Research Paper

Conventional and twin row spacing in different population densities for maize (*Zea mays* L.)

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The increase in maize yield is directly related to management practices, especially the manipulation of spatial arrangement of plants. This work aimed, evaluated the effect of conventional and twin-row spacing in different plant densities for maize, in the crop year 2011/2012. The treatments consisted of combinations between two rows spacing (twin-row spacing (0.2 x 0.7 m) and conventional spacing (0.7 m)) and five plant populations (50,000; 65,000; 80,000; 95,000 and 110,000 plants ha^{-1}). It was evaluated the plant height, insertion height of the first ear, stem diameter, number of rows, number of kernels per ear, weight of 1,000 kernels, ear weight and yield. The use of twin row spacing provided better results for stem diameter, number of rows, number of kernels per ear, weight of 1,000 kernels, ear weight and the average maize yield. The stem diameter, number of kernels per ear, weight of 1,000 kernels and ear weight responded negatively to increased plant density.

Key words: Plant arrangement, yield, agronomic characteristics.

INTRODUCTION

Maize (*Zea mays* L.) is one of the main cereals grown in Brazil, occupying increasingly significant positions on the value of agricultural production, especially in no-tillage system. With the increasing consumption, both for human and animal consumption and currently to meet the energy demand, there is a growing pressure to increase the yield of this kernel. According to the National Food Supply Company (CONAB, 2013), the estimated maize production in the crop year 2012/2013 will be approximately 77.45 million tons, with an average yield of 4,956 kg ha^{-1}, and 3.08% higher than the yield obtained in 2011/2012.

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Among the practices and techniques used to obtain higher maize yield, the choice of spatial arrangement of plants, resulting from the combination of row spacing and the number of plants per meter is one of the most important (Almeida et al., 2000). Recent researches have demonstrated that reducing spacing has contributed to increased yield (Gross et al., 2006; Modolo et al., 2010). Another option of plant arrangement that has been studied mainly in the United States refers to the twin-row or double row system, which is a form of plants distribution that seeks to increase the distance between the plants without affecting the phenotype and increase the yield by providing less competition in the row. Another possible advantage of double lines is to increase the number of plants per unit area without affecting the phytotechnical variables.

In Brazil, there is no record of studies and the utilization of double lines in grain crops, already in the United States, studies with double lines showed no consistent advantages in yield compared to the single-spaced, with same plant densities (Nelson and Smoot, 2009; Balkcom et al., 2011). For being a new form of plant arrangement, still no recommendation from sowing density and optimal spacing, since the optimum arrangement generally presents variation because is linked to the region at the time of sowing, the cropping system, the edaphoclimatic conditions and the genotype choice.

Studies developed by Cox et al. (2006) found that the row spacing (conventional or double row) did not affect plant height in both V_8 stage (vegetative phase with 8 developed leaves) as in the physiological maturity. However, the authors observed that the kernel yield was higher in the arrangement with double rows of maize compared to the arrangement using conventional rows. The authors attributed the results to the more rapid canopy closure, allowing better use of solar radiation and thus increasing the amount of photoassimilates accumulated in the stem. According to Nummer and Hentschke (2006), the rapid initial growth of the plant enables greater interception of solar radiation, and hence competitive advantages and higher photosynthetic rates, resulting in greater accumulation of photoassimilates.

In view of recently morphological changes introduced into maize genotypes, such as lower plant height, lower height of ear insertion, lower sterility of plants, shorter duration of the tasseling-silking subperiod and plants with more erect leaves; it turn necessary to reevaluate the recommendation of the spatial arrangement of plants (plant density, row spacing and row arrangement). Therefore, this study aimed to evaluate the effects of conventional and twin row spacing in different population densities for maize crops.

MATERIALS AND METHODS

The experiment was conducted in Pato Branco, Paraná State - Brazil, under field conditions in the summer crop of 2011/2012, on Hapludox soil according to the Brazilian Agricultural Research Corporation (EMBRAPA, 2006), with loamy texture (77.4% clay, 20.3% sand and 2.3% silt), and the following chemical characteristics (0.0 to 0.2 m), sampled before establishing the experiment: pH in $CaCl_2$ 6.0, 44.25 g dm^{-3} of organic matter, 25.02 mg dm^{-3} of P, and exchangeable cations: 326.49 cmol$_c$ dm^{-3} of K, 5.66 cmol$_c$ dm^{-3} of Ca, 3.72 cmol$_c$ dm^{-3} of Mg, 3.66 cmol$_c$ dm^{-3} of H+Al. The experimental area is located at 26°16'36" south latitude and 52º41'20" longitude west of Greenwich, with an average altitude of 769 m. The climate in the region is Cfa, according to Köppen climate classification (Caviglione et al., 2000), with average temperature in the coldest month below 18°C and above 22°C in the warmest month, with relatively warm summers, infrequent frosts and trend of concentration of rainfall in the summer months, but with no defined dry season.

The experiment consisted of a 2x5 factorial arrangement in a randomized block design with four replications, totaling 40 experimental units. The treatments involved the combinations of two spacing between the rows (conventional spacing and twin row spacing) and five levels of plant population (50,000; 65,000; 80,000; 95,000 and 110,000 plants ha^{-1}). Spacing adopted for twin rows was 0.20 m between double rows and 0.70 m between rows, that is, 0.2 x 0.7 m, and 0.7 m between rows for conventional spacing.

In the conventional spacing (0.7 m), each plot with 14 m^2 was comprised of four rows of 5 m length, and considered for evaluation only the two central rows. In the twin row spacing (0.2 x 0.7 m), each plot with 18 m^2 consisted of four double rows of 5 m length, and considered for evaluation the two central twin rows.

A single cross (SC) hybrid (P30F36 H) was used with semi-erect leaf architecture, temperate germplasm, early maturity, high proliferative capacity, high defensiveness against major diseases, and high-yield potential. Sowing was held on October 20, 2011 by hand with the use of manual seeder and equidistant pits according to the treatment. Two seeds were sown per pit, and when plants had four fully expanded leaves, the thinning was done in order to adjust the populations in each treatment. Seeds were treated with thiamethoxam systemic insecticide at 210 g a.i. per 100 kg of seed to prevent a possible attack by *Dichelops furcatus* and *Elasmopalpus lignosellus* (Zeller).

As topdressing fertilization was applied 600 kg ha^{-1} of the NPK formula 10-30-11, according to chemical characteristics of the soil and maximum expected yield of 13,000 kg ha^{-1} (SBCS, 2004). For broadcast topdressing, it was used 500 kg ha^{-1} of the formula 45-00-00 (Urea Plus) divided into two equal applications, the first application when plants had 3 fully expanded leaves, and the second, with 6 leaves. Additionally, it was applied 150 kg ha^{-1} of the formula 00-00-56 (KCl). The fertilization in the sowing furrows was performed with a seeder-fertilizer (Vence Tudo, model SA 14600) with a furrow opener mechanism.

During the cultivation, phytotechnical practices were performed according to the needs. In order to keep the crop free from weed competition, at preemergence, it was applied atrazine + simazine at 1,750 g a.i. ha^{-1}. For disease control, it was applied preventatively at pre-flowering a systemic fungicide picoxystrobin + cyproconazole at 80 g + 32 g ha^{-1} a.i.

The flowering was evaluated by the number of days, counted from the emergency, which 50% of the plants presented the inflorescence. In the stages V_6, V_{10} (vegetative phases with 6 and 10 developed leaves, respectively) and pre-flowering, gun-sprinkler irrigation was held with a water depth of about 20 mm and a shift irrigation of two hours, totaling 60 mm.

The following variables were examined: plant height - distance between the ground surface and the end of the male inflorescence; insertion height of the first ear - distance between the ground surface and the ear insertion; stem diameter - determined on the first internode above the plant base; number of rows per ear; number of kernels per ear, weight of 1,000 kernels and kernel yield. The variables plant height; height of the first ear and stem diameter

Table 1. Summary of analysis of variance for plant height (PH), first ear height (FEH), stem diameter (SD), 1,000 grain kernels (GK), ear weight (EW), number of rows (NR), number of grains per ear (NGE), and yield (YIE) of maize crop.

Sources of variation	GL	PH(m)	FEH(m)	SD(mm)	GK(g)	EW(g)	NR	NGE	YIELD(kg ha^{-1})
Conventional	---	2.34^{a}	1.41^{a}	23.00^{b}	358.05^{b}	152.85^{b}	17.5^{b}	577.70^{b}	$11,975.40^{b}$
Twin-row	---	2.34^{a}	1.42^{a}	24.00^{a}	370.65^{a}	164.85^{a}	18.1^{a}	640.20^{a}	$13,411.70^{a}$
Row spacing (E)	1	0.0003^{ns}	0.0003^{ns}	6.4000^{*}	1587.6000^{**}	1440.0000^{*}	3.6000^{*}	39062.5000^{**}	20629576.9000^{**}
Population (P)	4	0.0195^{ns}	0.0037^{ns}	22.7125^{**}	435.0250^{**}	8021.5875^{**}	0.7250^{ns}	5842.7875^{ns}	1530205.5375^{*}
E x P	4	0.0446^{ns}	0.0009^{ns}	2.7125^{ns}	79.8500^{ns}	31.3125^{ns}	0.4750^{ns}	1049.3125^{ns}	1565286.8375^{ns}
Residual	27	0.0218	0.0025	1.4111	89.5815	258.8667	0.7851	2998.1370	820570.9111
C.V (%)	---	6.32	3.52	5.09	2.60	10.13	4.98	8.99	7.14

Means followed by the same letter in the same column are not significantly different by Tukey's test at $p<0.05$ probability. * Significant at the $p<0.05$ level; ** Significant at the $p<0.01$ level; ^{ns}: not significant; CV: Coefficient of variation.

were determined in stage R_6 (physiological maturity), based on a sampling of ten plants collected in the working area of each plot. For the variables number of rows per ear; number of kernels per ear and weight of 1,000 kernels, were collected five ears in each plot at harvest. To quantify, the yield were used in only the two central rows of each experimental unit. Assessments involving weighing of the kernel were corrected to 13% moisture, and the manual maize harvest was performed at 186 days after sowing.

Data were subjected to the F-test of the analysis of variance. For row spacing, means were compared by Tukey test at $p<0.05$ probability. The effect of plant population was subjected to analysis of variance and regression. Models were chosen based upon the significance of the F-test at $p<0.05$ probability and on the coefficient of determination. Statistical analysis of data was performed using the software Sisvar for Windows 4.0 (Ferreira, 2000).

RESULTS AND DISCUSSION

The effect of spacing and plant population did not influence the time of inflorescence appearance (female flowering), since it occurred at 73 days after emergence (DAE) for all treatments.

Table 1 presents the summary of the analysis of variance and regression for biometric variables and yield components of maize crop. Row spacing had no significant influence on plant height and first ear height, however, influenced on stem diameter, 1,000 grain kernels, ear weight, number of rows, number of grains per ear, and yield. Plant populations influenced significantly only the stem diameter, weight of 1,000 kernels and ear weight. No significant interactions were detected between treatments.

The lack of effect of reducing row spacing on plant height and first ear height was probably because it had not increased expressively the intraspecific competition. Cox et al. (2006) analyzed a hybrid maize in double row (0.18 x 0.76 m) and conventional (0.76 m) spacing and observed that the spacing had no significant effect on plant height, in both V_8 stage as at physiological maturity, a result that agrees with the present work.

Demétrio et al. (2008) also observed no changes in plant height with reduced row spacing from 0.8 to 0.4 m, for early maturing hybrids (P30F80 and P30K73). However, Alvarez et al. (2006) observed an increase in plant height with reduced row spacing of 0.9 to 0.45 m from 0.9 to 0.7 m, respectively. The twin-row spacing provided a higher mean value of stem diameter (4.3%) compared with the conventional spacing (Table 1). This is probably due to the better interception of solar radiation in the crop canopy at early and before flowering stages. In the twin-row spacing, there is a more equidistant plant distribution, which provided the more rapid closure of the spacing by the plant canopy, thus using better the incident radiation and increasing the growth rate of maize plants at early stages (Nummer Filho and Hentschke, 2006), precisely when the stem diameter is defined (Fancelli and Dourado, 2004). The lowest value of stem diameter found in conventional spacing can be because plants are closer inside the row, enhancing the competition for nutrient, water and space.

There was a reduction in the stem diameter with increased density of plants (Figure 1), indicating that as it increases the population from 50,000 to 110,000 plants ha^{-1}, the stem diameter reduces approximately 20%. This effect is related to competition for resources and mainly by translocation of photoassimilates during kernel filling, especially at sites of high consumption or restriction of water. This result corroborates the assertion that the increase in population density

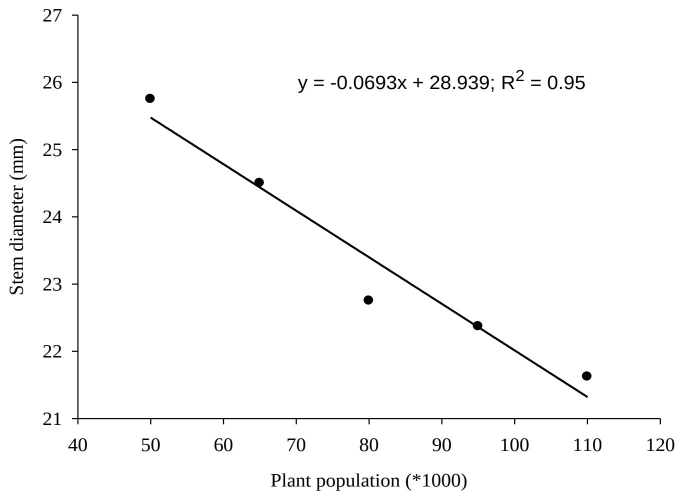

Figure 1. Stem diameter according to increased plant population in maize.

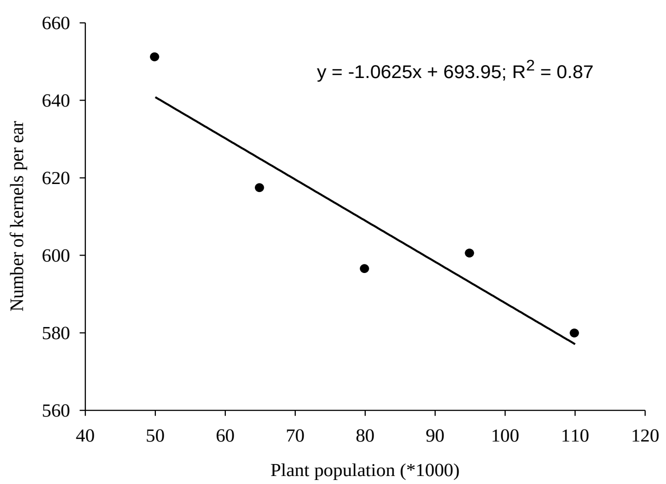

Figure 2. Number of kernels per ear according to increased plant population in maize.

decreases the stem diameter (Gross et al., 2006; Mendes et al., 2011). Only the row spacing influenced the number of rows per ear, with the highest value found in the twin row spacing (Table 1). Possibly, this result is due to the more even distribution of plants in this spacing.

According to Argenta et al. (2001) the reduction of spacing and the equidistant distribution of plants within the area significantly increased the number of rows per ear. However, this result differs from those obtained in studies with different row spacing. For Kappes et al. (2011), the row spacing did not affect the number of rows per ear of five maize hybrids with early and very early maturity when the spacing was reduced from 0.90 to 0.45 m. Piana et al. (2008) evaluated two maize hybrids from simple crossing (DOW 2B587; NB 4217) and four plant densities (55,000; 73,000; 91,000 and 110,000 plants ha^{-1}) and verified no difference in the number of rows per ear when reduced the spacing from 0.90 to 0.45 m.

The twin row spacing resulted in a higher number of kernels per ear (10.8%), compared with the conventional row (Table 1). This may have occurred because of more equidistant distribution of plants, which according to Sangoi (2001) and Argenta et al. (2001) enables the maximization of photosynthetic activity after anthesis, since plants spaced equidistant minimally compete for nutrients, light and other factors, favoring the better ear development.

In Figure 2, it is observed that the reduction on the number of kernels per ear with increased plant population, indicating enhanced competition for photoassimilates, which are necessary for reproductive growth. Similar results were reported by Amaral et al. (2005) who achieved a reduction on the number of kernels per ear with increasing density from 40,000 to 90,000 plants ha^{-1}. With respect to weight of 1,000 kernels, the twin row spacing showed a higher value (3.5%) compared with the conventional spacing (Table 1). This fact can be explained by the more equidistant distribution of plants in twin row spacing. This result corroborates with Nummer and Hentschke (2006) whose argues that the equidistant distribution of plants within the area improves yield components.

The plant population interfered significantly with the weight of 1,000 kernels, with a decreasing linear response to increasing plant density (Figure 3). This result is explained by the production capacity per plant, in general, at low densities, the individual production per plant is high, already at high densities the opposite occurs. According to Amaral et al. (2005) and Demétrio et al. (2008), the increased number of plants per unit area results in lower weight of 1,000 kernels, in other words; densities of 50,000 plants ha^{-1} allow heavier kernels than densities of 90,000 plants ha^{-1}.

Different row spacing significantly influenced the ear weight, and for the twin line spacing, the observed mean was 164.85 g, 7.8% higher than the mean of the conventional spacing (Table 1). The highest ear weight found for twin line spacing is justified by the equidistant distribution of plants and the largest stem diameter resulting from this treatment. The effects of plant population on the ear weight (Figure 4) were similar to those found in the weight of 1,000 kernels, that is, the 120% increase in the population, from 50,000 to 110,000 plants ha^{-1} led to a reduction of 67.3% in ear weight. Borghi et al. (2004) also observed a reduction in the ear weight given the increase in the number of plants when compared three forms of cultivation (conventional, minimum tillage and no-tillage) and three plant densities (55,000; 65,000 and 75,000 plants ha^{-1}) and two forms of fertilization (incorporated and on the surface) to the hybrid P 30F33.

The twin row spacing resulted in 12% increase in the average yield of maize compared with the conventional

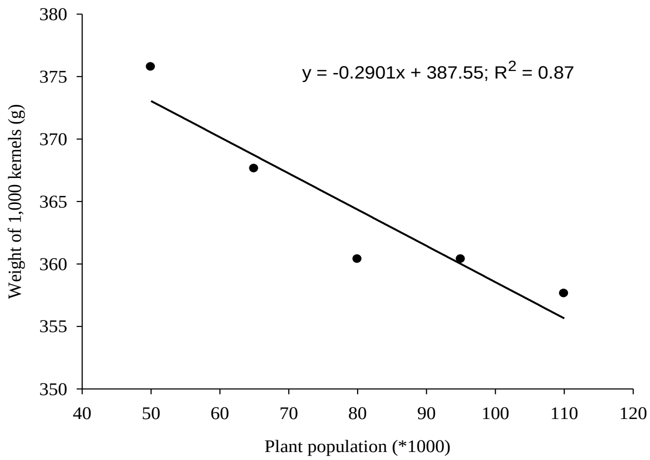

Figure 3. Weight of 1,000 kernels according to increased plant population in maize.

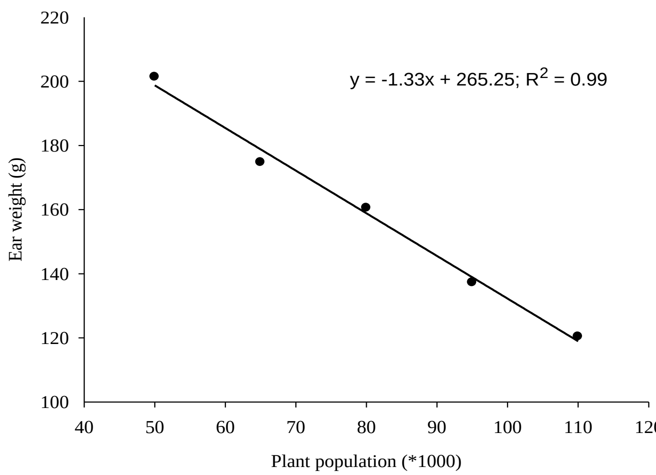

Figure 4. Ear weight according to increased plant population in maize.

spacing (Table 1). The increase in kernel yield in this spacing can be attributed to greater efficiency in radiation interception and decreased competition for light, water and nutrients between plants in the row, due to the more equidistant distribution (Nummer and Hentschke, 2006; Demétrio et al., 2008). Balkcom et al. (2011) also reported yield advantage in maize when used twin row spacing (0.19 x 0.76 m) compared with the conventional row spacing (0.76 m). However, Robles et al. (2012) by assessing three maize hybrids for three consecutive years in twin row spacing (0.20 x 0.76 m) and conventional spacing (0.76 m) at four densities (69,000; 81,000; 93,000 and 105,000 plants ha^{-1}), found that the maize kernel yield with twin row spacing was not significantly higher than conventional spacing for all hybrids and plant density used.

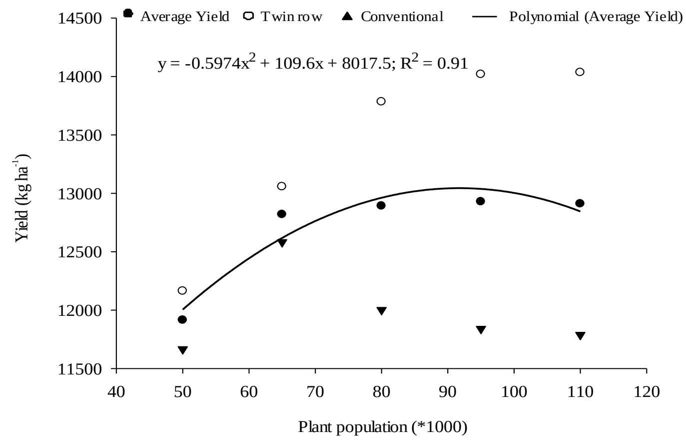

Figure 5. Maize yield according to increased plant population.

The highest yield of 12,950 kg ha^{-1} was found with 91,700 plants ha^{-1} (Figure 5). Recent studies showed positive responses to increased maize yields by increasing plant population, with maximum yield achieved with densities close to 90,000 plants ha^{-1} and decreasing at higher populations, close to 100,000 plants ha^{-1} (Marchão et al., 2005; Nummer and Hentschke, 2006). Although yield showed no significant interaction between treatments (Table 1), there is a distinct behavior at high plant densities between the different spacing. The twin row spacing maintained high yields when adopted plant population above 65,000 plants ha^{-1}. In turn, in the conventional spacing, when the number exceeded 65,000 plants ha^{-1}, the yield decreased. Therefore, the data indicate a trend that in the twin row spacing it would be possible to increase the number of plants per unit area.

The results show that in maize crop, there is a strong relationship between yield and number of harvested ears. This is because the yield components, weight of 1,000 kernels, number of kernels per ear, and ear weight responded linearly, decreasing as the yield responded positively to increased plant population.

Conclusions

The use of twin row spacing provided increment in stem diameter, number of rows per ear, number of kernels per ear, weight of 1,000 kernels, ear weight and average yield of maize crop in relation to conventional row spacing. The stem diameter, number of kernels per ear, weight of 1,000 kernels, and ear weight responded negatively to increased plant density. The density to achieve maximum production is 91,700 kg ha^{-1}, with a tendency to obtain higher yields with larger populations using twin row spacing than with conventional spacing. The hybrid P 30F36H has adaptability to high plant densities and the use of twin row spacing, for the climatic conditions of Pato Branco, Paraná State.

Conflict of Interests

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

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Full Length Research Paper

Soil evaporation under different straw mulch fractions

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This study was conducted at the Agronomic Institute of Parana (IAPAR), Londrina, Parana state (latitude 23° 18' S, longitude 51° 09' W, average elevation of 585 m). Londrina's climate in Köppen's classification is Cfa type, that is, humid subtropical climate with rain in all seasons and droughts in winter. Soil water evaporation (E) was determined for different soil mulching cover fractions. The treatments were applied on 2.66 m^2 x 1.3 m deep weighing lysimeters for the determination of E by mass difference with an accuracy of 0.01 milimeter in 1 h intervals. Evaporation was determined for different mulching fractions using the average amount of wheat straw produced in that region of 5 $ton.ha^{-1}$ distributed onto 25, 50, 75 and 100% of the soil surface. In the first cycle (28 October to 27 November, 2008), the reductions of E in treatments with 25, 50, 75 and 100% mulching cover in relation to the not-mulched soil were 15, 17, 20 and 30%, while in the second cycle (19 January to 16 February, 2009), the reduction were 15, 30, 45 and 60%, respectively.

Key words: Wheat straw, mulching uniformity, soil water loss.

INTRODUCTION

One of the most important ways to loss water in the fields is the Evapotranspiration (ET). The ET is the sum of Transpiration (T) of the plants and Evaporation (E) from soils. We cannot change the T of plants mainly because how much more the plant transpires larger is their production. But we can control the losses from the soil covering it. The cropped fields with mulching is one of the best practices to increase the storage of water in the soil to plants usage. Mulching reduces soil surface for water evaporation in No Tillage System (NTS) as compared to bare soil. However, due to the higher water storage capacity and greater soil moisture content, evaporation cycle in NTS takes longer than in the conventional tillage system (Dalmago et al., 2004).

The Parana State is the second biggest producer of soybean in Brazil and the second harvest is the wheat. Then the soybean is cultivated over the wheat straw. In this state, the wheat produces around 5 $ton.ha^{-1}$ of straw, but the most important is not the quantities, but is the uniformity, because if you have no covered soil your E will be greater. The ET and soil moisture estimated based on meteorological models have the advantages of ease calculation and possibility of application on different regions; however, model calibration is required using field

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measurements.

Prevedello et al. (2005) worked on numerical modeling of soil water evaporation and succeeded in simulating one-dimensional soil water evaporation process, both in porous material using stratified sandy and in clayey material in columns with distinct layer thicknesses and hydraulic properties. Nevertheless, soil water evaporation models under mulching are not available and the available estimation methods depend on field measurements under culture. Medeiros et al. (2003) presented a detailed report on the estimation of reference Evapotranspiration (ETo) using Penman-Monteith FAO's equation for lysimetric measurements and empirical equations and evaluated the main methods: Thornthwaite, modified Thornthwaite, Class A Tank, Hargreaves and Samani and Priestley and Taylor. According to them, the lysimetric measurement of ETo has an average performance in relation to estimates by the Penman-Monteith method, suggesting that equipment use must be investigated. Among the evaluated ETo estimation methods, Priestley and Taylor and modified Thornthwaite methods, the ones with local adjustment, performed better on a daily scale. The methods with the worst performance were Thornthwaite, Class A Tank and Hargreaves and Samani.

The functional model developed by Ritchie (1972) calculates soil surface evaporation (Es) and plant transpiration (Ep). This model takes into account the soil surface water evaporation phases described by Philip (1957) in the calculation of Es. As a result, Phase 1 is considered when the soil surface is wet and Es occurs at a constant rate, being limited only by ETo. In Phase 2, the soil surface is considered to be dry and Es depends on the soil physical parameters. In Phase 3, the soil is considered to be very dry and does not afford minimal conditions for culture development. This phase is not considered for irrigation purposes. To define Es in Phase 1, Ritchie (1972) established the parameter U, and for Phase 2, the parameter α. Both parameters can be determined with weighing microlysimeters, as described by Rodrigues et al. (1997).

Stone et al. (2006) observed that mulching in appropriate amounts is very important in irrigated agriculture because it alters the soil-water ratio, prevents evaporation and reduces the crop evapotranspiration rate, especially in the stage when the canopy does not fully cover the soil. The result is a reduction in the frequency of irrigation and a cost reduction in irrigation system operation.

Veseth et al. (2007) reported that the use of mulching distribution in wheat crop has increased mainly due to the increase in the cutting width of combine harvesters and the use of taller wheat cultivars. Combine harvesters with 9.14 m cutting platforms or longer are already commercially available; however, most equipment is not equipped to spread large volumes of phytomass uniformly.

For soybean crop the situation is not different. Kunz et al. (2008) investigated innumerous soybean combine harvesters and observed irregular mulch distribution, regardless of cutting width. They also reported that irregular mulching results in irregular nutrient availability to plants, with larger amounts available in harvester-mulched strips. They pointed out the importance of a trash sieving distribution system for a straw distribution closer to the ideal. Pereira et al. (2002) investigated the effect of 25, 50, 75 and 100% mulching on irrigated bean crops and showed that significant economy with a reduction in the number of irrigations can be achieved starting with 50% mulching. However, crop yield was not significantly by mulching. The objective of this study was to determine soil water evaporation in bare soil under different wheat straw mulching cover fractions in the region of Londrina, Paraná state, Brazil.

MATERIALS AND METHODS

This study was performed at the Agronomic Institute of Paraná (IAPAR) in Londrina, Paraná state (latitude 23° 18' S, longitude 51° 09' W, average elevation of 585 m). Londrina's climate in Köppen's classification is Cfa type, that is, humid subtropical climate with rain in all seasons and the occurrence of droughts in winter. The mean temperature in the warmest month is over 22°C and in the coldest month, below 18°C. The soil is Eutrophic Red Latosol, according to Embrapa (2006), with the following granulometric composition: 6% sand, 15% silt and 81% clay.

Soil water evaporation with different mulching fractions and in fallow land was determined in ten weighing lysimeters, installed at IAPAR, close to the SIMEPAR meteorological station. These lysimeters have impermeable metallic tanks measuring 1.4 m wide, 1.9 m long and 1.3 m high, filled with local soil, leveled with the soil surface on a scale, with a lever system to reduce the mass. Was made only two replications for each treatment because weighing lysimeters are expensive equipments. However, weighing lysimeters have a very good precision and can be safely use the average. Mass variations equivalent to 0.1 milimeter water depth (mm) were determined in intervals of up to 1 h with a load cell that produces electric signals with a sensibility of 2 ± 10% $mV\ V^{-1}$ and an accuracy of 0.02% of end of scale value. Readings were recorded every 3 s, totaling 200 measures in 10 min. The data acquisition system stores the interval mean value to avoid variations, especially caused by wind. The electric signal is transferred from the data acquisition system to a computer by a digital interface and converted to mass unit and then to depth unit (mm) with the lysimeter initial calibration equations.

The location of the lysimeters close to the SIMEPAR meteorological station allowed the comparison of the lysimeter data with the reference evapotranspiration (ETo) calculated from the station data. Data were collected every 10 min with a data logger and transferred to a computer for calculation of the lysimeter mass variation. Measurements were also taken upon draining. Soil water evaporation of bare soil and of soil mulched with 5 $ton.ha^{-1}$ distributed according to four mulch fractions (25, 50, 75 and 100%) was determined in two observation cycles, from 28 October to 27 November, 2008 (cycle 1) and from 19 January to 16 February, 2009 (cycle 2). Wheat straw was randomly collected in the field after cropping and dried in a circulating air, oven for 48 h at 65°C. Straw moisture in field conditions was determined before division into four volumes of 1.33 kg, the equivalent to 5 $ton.ha^{-1}$ of dry matter, which were applied in 25, 50, 75 and 100% fractions in

Figure 1. Wheat straw distribution simulating 25, 50, 75 and 100% soil mulching, bare soil.

separate lysimeters, as shown in Figure 1. ETo was calculated by the Penman-Monteith - FAO method (Allen et al., 2006) using the daily data from the SIMEPAR automatic meteorological station, Londrina, Paraná, with Equation (1):

$$ETo = \frac{0.408\Delta(Rn-G) + \gamma\frac{900}{(T+273)}U_2(e_s - e_a)}{\Delta + \gamma(1+0.34U_2)} \quad (1)$$

In which,

ETo - reference evapotranspiration, mm d^{-1}, Rn - net radiation at the crop surface, MJ m^{-2} d^{-1}, G - soil heat flux density, MJ m^{-2} d^{-1}, T - mean air temperature at 2 m high, °C, U2 - wind speed at 2 m high (m s^{-1}), es - saturation vapor pressure, kPa, ea - actual vapor pressure, kPa,Δ - slope vapour pressure curve, kPa °C^{-1}, γ - psychrometric constant, kPa °C^{-1}.

Soil water evaporation coefficient (K_e) was determined from daily evaporation values measured in the weighing lysimeters. According to Allen et al. (2005), when the soil surface is fully wet by rainfall or irrigation, the evaporation is only determined by the energy available for evaporation. This corresponded to 100% of the lysimeter area, as there were no plants. Allen et al. (2005) proposed the equation where the Ke value can be calculated with Equation (2).

$$K_e = \frac{E}{ETo} \quad (2)$$

In which,

K_e - evaporation coefficient, dimensionless, ETc - crop evapotranspiration, mm d^{-1}.

RESULTS AND DISCUSSION

Daily evaporation values

The first cycle started with 20 mm irrigation of all treatments at 08:00 A.M (Figure 2). The not-mulched and 25% mulching treatments had higher evaporation than the ETo. During 2 November, 2008 it was cloudy and rainy all day long with higher intensity at noon and 11 November, 2008 started with high humidity, which led to a high E in all treatments. On 4 November, 2008, 10 mm rain started in late afternoon and lasted until few of next day. However, evaporation was not very high because the weather was partially cloudy. On the following day, 46 mm rain lasted all day long with cloudy sky. As 7 November, 2008 started cloudy, humidity was high and, therefore, was evaporation was similar for all treatments. On 11 November, 2008 it rained again from 12:00 P.M to 02:00 P.M, 32 mm. That reduced ETo, as it remained cloudy during the time of greater irradiation. 10 November, 2008 had high evaporations values, as can be observed in Figure 2, due to the rainy day. On 11 November, 2008, it rained 16 mm until 10:00 P.M, which

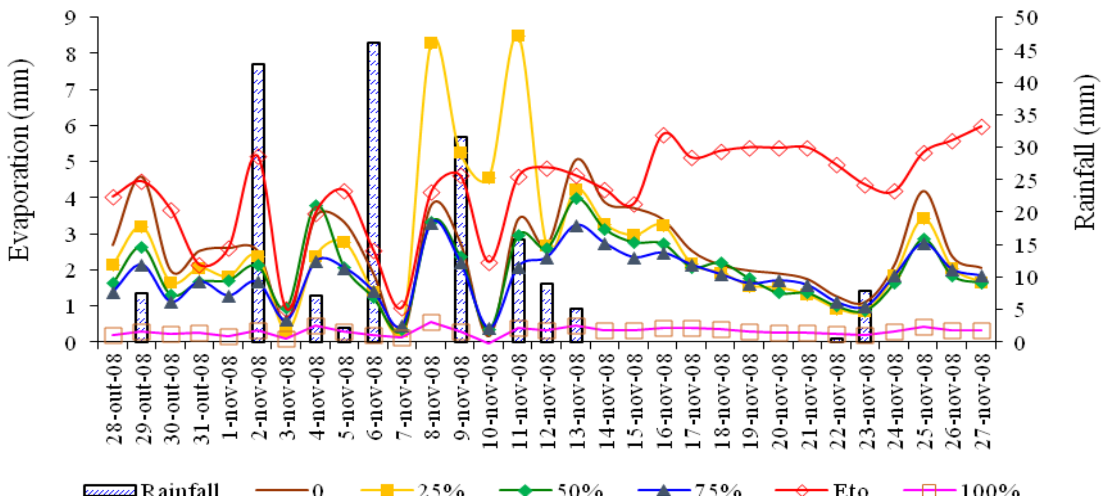

Figure 2. Soil evaporation from lysimeters with different mulching fractions (0, 25, 50 and 100%) during the first cycle (28 October to 27 November, 2008).

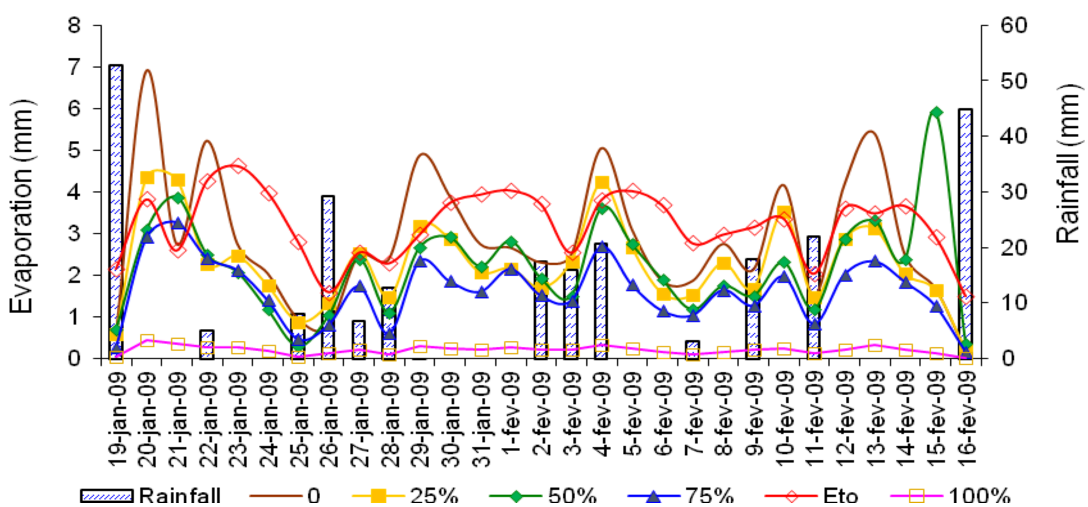

Figure 3. Soil evaporation from lysimeters with different mulching fractions (0, 25, 50 and 100%) during the second cycle (19 January to. 16 February, 2009).

raised the E of the following day. On 12 November, 2008, it rained at night, but less than 9 mm, and also on 13 November, 2008, 5 mm.

The dry period that followed made the effect of the treatments evident. As the soil dried, evaporations decreased. Therefore, the treatments which retained most water in the soil had greater evaporation. It rained a little at 10:00 A.M on 21 November, 2008 and in the late afternoon of 22 November, 208, which did not alter the value of evaporation of the treatments. On 23 November, 2008, it rained 8 mm at 03:00 P.M, which increased the evaporation value of the bare not-mulched soil substantially. Figure 3 shows soil evaporation for the second cycle.

Due to high soil moisture, bare soil water evaporation was greater than the ETo, since water at soil surface evaporated freely. This was evident during early in the morning period. According to Figure 3, on 19 January, 2009, the day remained cloudy and it rained nearly all day, reaching 53 mm. 1st January, 2007 started with high humidity and E was even greater than ETo. The same happened on 22 January, 2009, with some rain (5 mm) at 09:00 A.M On 25 January, 2009, rain started at 06:00 P.M, which continued on the whole of 26 January, and let up only in the morning of 27 January, 2009. Besides starting with high humidity, on 27 January, 2009 it rained at 10:00 A.M, 01:00 P.M and 07:00 P.M. Rain started in the small hours of 28 January, 2009 and stopped at

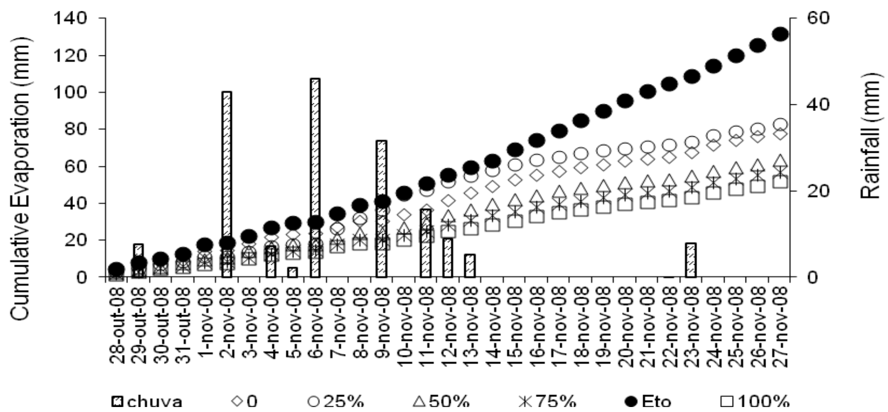

Figure 4. First cycle of soil water evaporation.

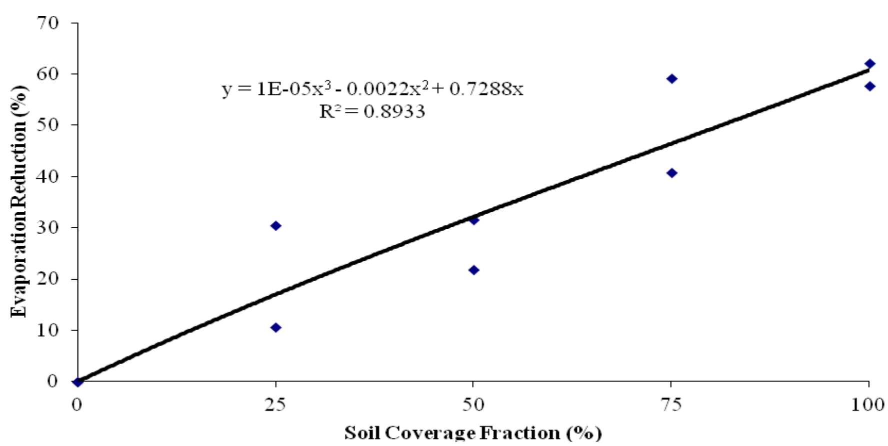

Figure 5. The reduction of soil water evaporation in the first cycle.

09:00 A.M., and started again at 07:00 P.M, giving a total rainfall of 58 mm. As a result, the not-mulched soil evaporation was greater than the ETo again on the next day.

On 02 February, 2009, rain started at 11:00 P.M and lasted until the next morning. However, 3 February, 2009 was partially clouded with a little rain at 03:00 P.M. In the small hours of 04 February, 2009, it rained again and it let up at 07:00 A.M. Total rainfall for those three days was 54 mm, which increased soil moisture in the surface layer and incremented soil evaporation, particularly for the not-mulched treatment, followed by the treatments with lower mulching fraction. In addition, a low rainfall event (3 mm) occurred in the late afternoon on 07 February, 2009, increasing evaporation again for the treatments with less percentage of mulching fraction. Late at night on 9 February, 2009, it rained approximately 20 mm, which raised the next day's evaporation again at amount above ETo. 11 February, 2009 was partially clouded, with rain all day long and a total rainfall of 20 mm, which was sufficient to make the not-mulched soil evaporation higher than the ETo on the next two days. 16 February, 2009 ended clouded with a total rainfall of 45 mm. Cumulative raifall, for the first cycle reached 178.2 mm however, it was concentrated in the middle of the period. One can observe a small difference between treatments with 0 and 25% as well as 75 and 100% mulching (Figure 4).

The reduction in evaporation in the first cycle for the 25% mulching treatment was approximately 15%, while for 50, 75 and 100% mulching, it reached 30, 45 and 60%, respectively. Figure 5 correlates the cumulative evaporation in the first cycle with its percent reduction in the not-mulched soil. Considering the values given in Figure 5 in relation to ETo, the percent reduction values found for the first cycle were 15, 30, 45 and 60% for the bare soil, and with 25, 50, 75 and 100% mulching, respectively. Similar results were found by Xie et al. (2006), who reported a soil water evaporation of 40.7% during the watermelon culture cycle without mulching and 17.8 for 25% soil cover with sand and gravel in mild climate conditions with maximum annual temperature of

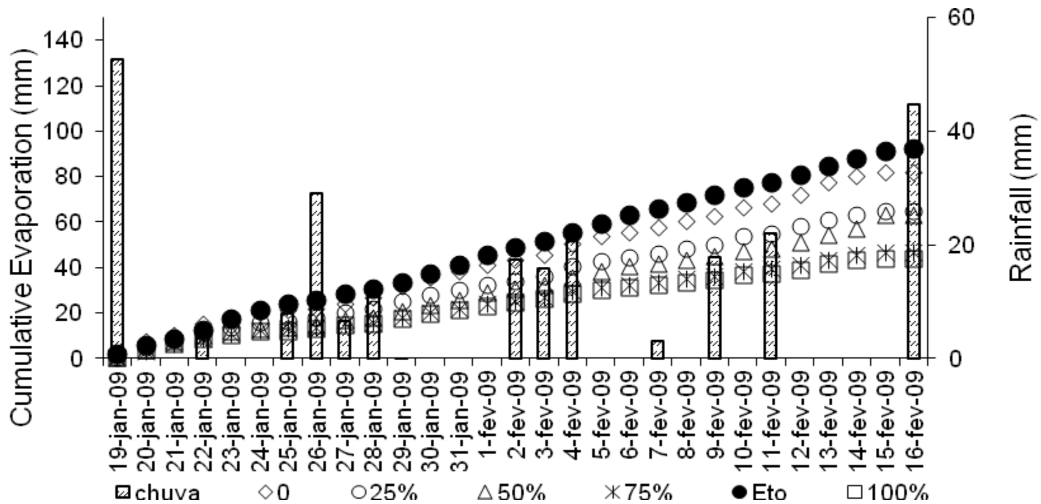

Figure 6. Second cycle of soil water evaporation.

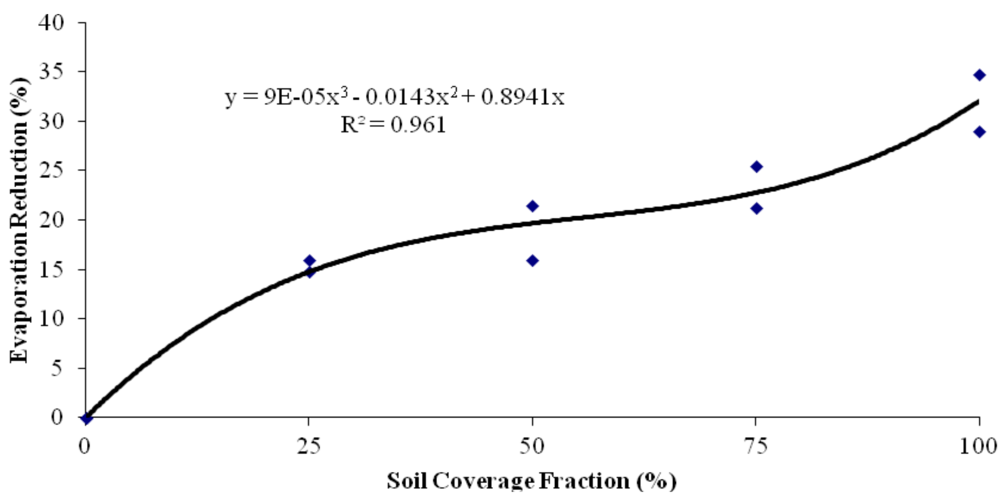

Figure 7. The reduction of soil water evaporation in the second cycle.

20.7°C. In the second cycle, accumulated rainfall was 256.5 mm, in contrast to the first cycle with 178.2 mm, gave a difference of nearly 80 mm. Furthermore, in the second cycle, rainfall was more uniformly, with a greater number of rainfall events in more regular intervals during the period (Figure 6). Thus, evaporation decreased in a more uniformely mulching distribution treatment, corroborating with Andrade et al. (2002). The reduction in evaporation for the 25% mulching treatment was approximately 15%, while for 50, 75 and 100% mulching, it reached 17, 20 and 30%, respectively (Figure 7).

Evaporation coefficient (Ke)

The FAO 56 approach is based on the combination of the concepts of reference evapotranspiration (ETo) and "single" and/or "dual" Kc (Allen et al., 2006). The "single" Kc combines the effects of water soil evaporation and crop transpiration together, while the "duale" Kc method, combines the basal crop coefficient (Kcb) and the soil water evaporation coefficient, Kc (Allen et al., 2006). Thus, when a more accurate estimate of the actual crop hydric demand is necessary, the dual Kc method recommended is as Kc decomposed into two components. The calculation of the dual Kc allows a greater accuracy, since Kc varies greatly with the meteorological elements and the surface soil moisture, especially in the rainy season and total area irrigation.

In this study, the soil was kept fallow and Ke was obtained as the ratio of the soil water evaporation in the treatments and the ETo. The Ke values of the soil

Table 1. Ke values for five soil mulching fractions.

PF1						PF2					
Date	%					Date	%				
	0	25	50	75	100		0	25	50	75	100
10/28/2008	1.0	0.8	0.6	0.6	0.5	01/19/2009	0.3	0.3	0.2	0.1	0.2
10/29/2008	0.5	0.5	0.4	0.4	0.5	01/20/2009	1.8	1.2	0.8	0.6	0.6
10/30/2008	1.2	1.2	0.9	1.0	0.9	01/21/2009	1.1	0.8	1.2	1.3	1.4
10/31/2008	1.0	0.8	0.8	0.5	0.4	01/22/2009	1.2	0.8	0.7	0.6	0.4
11/01/2008	0.5	0.6	0.5	0.4	0.4	01/23/2009	0.6	0.5	0.5	0.5	0.3
11/02/2008	0.6	0.1	1.7	0.4	0.8	01/24/2009	0.5	0.3	0.3	0.3	0.3
11/03/2008	1.0	0.7	1.5	0.7	1.0	01/25/2009	0.3	0.3	0.1	0.1	0.0
11/04/2008	0.8	0.8	0.5	0.5	0.4	01/26/2009	0.5	0.9	0.8	0.9	0.6
11/05/2008	0.7	0.4	0.6	0.6	0.3	01/27/2009	1.0	1.0	0.9	0.6	0.5
11/06/2008	0.2	0.1	0.3	0.4	0.6	01/28/2009	1.0	0.7	0.7	0.3	0.3
11/07/2008	0.9	0.9	0.8	0.9	0.9	01/29/2009	1.6	0.9	0.7	0.8	0.7
11/08/2008	0.6	0.6	0.4	0.4	0.3	01/30/2009	1.0	0.8	0.6	0.5	0.4
11/09/2008	0.1	0.2	0.1	0.2	0.1	01/31/2009	0.7	0.6	0.4	0.4	0.3
11/10/2008	0.7	0.6	0.7	0.4	0.5	02/01/2009	0.7	0.5	0.5	0.6	0.4
11/11/2008	0.6	0.5	0.5	0.5	0.4	02/02/2009	0.6	0.4	0.3	0.4	0.4
11/12/2008	1.1	1.0	0.9	0.6	0.5	02/03/2009	1.0	0.8	0.6	0.5	0.5
11/13/2008	0.9	0.7	0.7	0.6	0.4	02/04/2009	1.3	1.1	1.0	0.6	0.5
11/14/2008	1.0	0.8	0.7	0.6	0.5	02/05/2009	0.8	0.7	0.5	0.4	0.4
11/15/2008	0.6	0.6	0.4	0.4	0.4	02/06/2009	0.5	0.4	0.4	0.3	0.2
11/16/2008	0.5	0.4	0.4	0.4	0.5	02/07/2009	0.7	0.5	0.4	0.3	0.2
11/17/2008	0.4	0.4	0.4	0.3	0.4	02/08/2009	0.9	0.7	0.6	0.5	0.1
11/18/2008	0.4	0.3	0.3	0.3	0.3	02/09/2009	0.7	0.6	0.6	0.4	0.5
11/19/2008	0.4	0.3	0.2	0.3	0.3	02/10/2009	1.2	1.0	0.6	0.5	0.5
11/20/2008	0.3	0.2	0.2	0.3	0.3	02/11/2009	0.7	0.8	0.6	0.3	0.3
11/21/2008	0.3	0.2	0.2	0.2	0.3	02/12/2009	1.2	0.8	0.7	0.5	0.3
11/22/2008	0.3	0.2	0.2	0.2	0.3	02/13/2009	1.5	1.0	0.7	0.7	0.5
11/23/2008	0.5	0.5	0.4	0.5	0.4	02/14/2009	0.7	0.6	0.5	0.5	0.3
11/24/2008	0.8	0.7	0.5	0.5	0.5	02/15/2009	0.6	0.6	0.3	0.4	0.2
11/25/2008	0.4	0.4	0.3	0.4	0.3	02/16/2009	0.1	0.2	0.1	0.2	0.0
11/26/2008	0.3	0.3	0.3	0.3	0.3						
11/27/2008	0.3	0.6	0.4	0.2	0.6						

mulched with wheat straw in the two cycles are given in Table 1. Table 1 shows that Ke increased significantly after rain, being greater than 100% in many occasions in all treatments, with the exception of the 100% mulched soil, which had a maximum of 80% once. This was due to the high soil surface moisture, which contributes to increase evaporation. The 100% mulching treatment is the only one that poses a physical barrier all over the area and avoids greater moisture losses.

Similar results found for the second cycle, with higher increase in Ke for the soil with 100% mulching cover because of its previous stabilization or because evaporation was over. However, for the other treatments, Ke increased to values far higher than that of the 100% mulched soil. In treatments with 3 and 6 $ton.ha^{-1}$ of corn residues, Andrade et al. (2007) found maximum Ke values of approximately 50% of those of bare soil, with values of 0.70 and 0.44, respectively. Table 1 presents the daily Ke values for the two periods and different soil mulching fractions and shows that the Ke values were greater than 1.0 in some days despite the rain during the experimental period, as shown in Figures 4 and 6.

Mutziger et al. (2005) compared the results of the model presented in report FAO-56 with soil water evaporation values measured in a lysimeter. The Ke values found in this study after rain, when the values were greater than 1.0, corroborated those values. However, Snyder et al. (2000) found Ke values in the range of 0.61-0.90 in a study carried out in California. Oliveira et al. (2003) also determined the crop coefficient for a carrot culture using the method proposed in report FAO 56. The Ke values were greater than 1.0 in the culture initial phase after rain or irrigation. Oliveira Neto et al. (2011) concluded that evapotranspiration in mulched soil beetroot culture was 53% smaller than in non-mulched soil due to the decrease in soil water evaporation, mainly in the beginning of the cycle. Gava et al. (2013) observed that increasing densities of wheat straw decreased soil water evaporation in relation to not-mulched soil. Ke increased immediately after rain, that is, with the increase in the soil moisture, which corroborates the results obtained by Andrade et al. (2002).

Conclusions

Relative to bare soil, the soil evaporation rate can be reduced around, 15% for the 25% of mulching and can vary from 17 to 30%, from 20 to 45% and 30 to 60% due to the wheat straw soil mulching fractions of 50, 75 and 100%, respectively. This variation is mainly due to different rainfall patterns and what time of the day it occurs. Its possible to reduce the evaporation from soil until 60% using a management with wheat straw.

Conflict of Interests

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

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Variation in yield component, phenology and morphological traits among Moroccan bitter vetch landraces *Vicia ervilia* (L.) Wild.

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In this study, 19 ecotypes of *Vicia erv*ilia (L.) Willd. sampled in the traditional agro ecosystems of the Rif mountains (Northwestern of Morocco) were investigated for their characteristics related to germination, phenology, morphology and yield. A large variation was determined among these traditional populations, especially among productive capacity traits as harvest index, which variation can reach up to 40%. The extent of phenotypic variation suggests an important genetic diversity. Indeed, despite a limited geographical scale, we have highlighted population differentiation linked to the production basin origin. Moreover, these populations are characterized by a short lifespan, the absence of dormancy together with precocity, which give to these landraces an interesting adaptive potentiality in the search for alternative crops tolerant to aridity and temperatures raises predicted by ongoing global changes.

Key words: *Vicia ervilia,* landraces, Rif, ecotypes, genetic resources, alternative crops.

INTRODUCTION

Vicia ervilia (L.) Willd. is an ancient legume whose domestication starting during the Neolithic and the Bronze Age, in the eastern Mediterranean, in Anatolia and northern Iraq (Zohary and Hopf, 2000). In the western Mediterranean its presence has been reported in various archaeological sites in the Iberian Peninsula and northern Morocco (Zapata et al., 2004; Pena-Chocarro et al., 2013). It is part of Mediterranean species that have been marginalized since the 16th and 17th century with the introduction of new crops from the New World (Hernandez-Bermejo and Gonzales, 1994). Currently, it is a minor crop grown around the Mediterranean basin and

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the Near East. It is distributed in southern of Europe, west and central Asia and Northern Africa (GRIN, 2008). In the Mediterranean region the overall socio-economic transformations and their impacts on agricultural production systems have been shown to result in real risk of genetic erosion of the diversity of several crops species, including *Vicia* (IBPGR, 1985).

V. ervilia world production is estimated around 800,000 tons per year with a mean yield of 1600 Kg/ha (FAO, 2013). The primary use of the bitter vetch grains is cattle feeding (Enneking, 1995; Francis et al., 1999), recent studies recommended it as protein and energy source (Abdullah et al., 2010). Indeed, seeds contain up to 22 to 28.5% of protein, 18,196 MJ / kg of gross energy and 12.967 MJ / kg of metabolic energy (Fernandez-Figares et al., 1993; Aletor et al., 1994; Yalcin and Önol, 1994; Farran et al., 2005; Sadeghi et al., 2009a). It can also be used as a fodder plant (Abd El-Moniem et al., 1988; Turk, 1999). Its use in human food is rare due to its toxicity (Sadeghi et al., 2009b) and it has only been reported during starvation periods (Enneking et al., 1995).

In Morocco, it is a minor crop in the northern part of the country where it is cultivated in the regions of Aarbaoua, Ouazzane, Pre-Rif, Rif, Fez and Taza (Foury, 1954), but its cultivation has declined for decades. Indeed, the cultivated areas were already stagnating in the 80s and 90s around 20,000 ha (Bounejmate, 1997) reaching nowadays 10,000 ha (FAO, 2013). The varieties used are local populations maintained by traditional farming practices within traditional agro-ecosystems of the Rif Mountain (Hmimsa and Ater, 2008; Ater and Hmimsa, 2008). This local selection resulted in ecotypes adapted to the local agro-climatic conditions (Foury, 1954; Francis et al., 1994; Enneking et al., 1995; Bounejmate, 1997) whose genetic resources assessment has nearly never been done (Francis et al., 1994; Van de Wouw et al., 2001) for limited *Vicia* studies.

In accordance with previous expectations (FAO, 2006; IPCC, 2007), the Mediterranean climate regions have experienced perceptible temperature rise in temperatures and periods of severe drought under climate change effect during the last decades (Bindi and Olesen, 2011; Supit *et al.,* 2010). Thereby, rainfed agriculture is now facing high risks and uncertainties (Trnka et al., 2011). Northern Africa is one of these endangered areas that might undergo a 20% rainfall decrease and an increase of annual mean temperature reaching 0.2°C per decade (Nefzaoui et al., 2012). Such environment require various strategies in operating systems and agricultural policies to cope with these threats. Among the productions of economical importance, forage production and animal feeding, are vulnerable strategic sectors to these changes (Hopkins, 2012). Within this frame the exploration of alternative crops has become a priority (López-I-Gelats and Bartolomé, 2012). Selecting forage species adapted to climate change requires the evaluation of plant genetic resources for species that display a high tolerance to drought and high temperatures. Among these candidate species, priority should be given to ancient locally grown species, supposedly more adapted to local conditions (Berger et al., 2002; Van de Wouw et al., 2001) rather than introducing new crops. Among the cultivated species that belong to the genus *Vicia*, *V. ervilia* is a very promising species with good adaptive abilities, especially tolerance to aridity, that can be cultivated with low rainfall level (Foury, 1954; Maxted, 1995; Abd El-Moneim and Saxena, 1997). It is a short life-cycle species; it can be used for both grain production or forage, and show good nutritional quality. Hence the goals of this study were to provide a first assessment of morphological diversity, phenology variations and components of productivity among local populations of *V. ervilia* in Morocco. This is a relevant contribution to the assessment of the productive potential and diversity of this species, in a goal of its valorization.

MATERIALS AND METHODS

Plant material

A survey was conducted in the main principal growing area of *V. ervilia* in Morocco, located in the Tingitane peninsula, in the western Rif (Figure 1). Seeds of 19 populations distributed between different regions were sampled directly from the field (Table 1). The seeds were sampled from individuals randomly chosen along a diagonal line crossing the fields. In parallel, surveys were conducted with farmers to determine the origin of the seeds sown and circuit of exchanges. On average 5 farmers are interviewed by locality.

Experimental device

For each population, 30 seeds were used for germination test. The seeds are disposed in petri dishes, imbibed with distilled water and placed in the dark. Germination time (GT), precocity (PR) and percentage of germination (PG) were noted. Plants used for phenotypical measures were grown in a greenhouse in pots with potting soil as a substrate. For each population, ten replicates were sown (10 pots) with one plant per pot. The device was then composed of 190 randomized pots. Twenty quantitative characters related to morphology, phenology and production were measured (Table 2). Harvest index (HI) was calculated by dividing seed yield (SY) by the total biomass (BY) multiplied by 100.

Used software

Descriptive statistics and analysis of variance (ANOVA) were performed by SPSS Statistics (17.0). The principal component analysis (ACP) was performed by XLSTAT (13.1). The location map of the surveyed population was carried out by using the DIVA-Gis software (5.2).

RESULTS AND DISCUSSION

Seed origin

87.5% of the farmers we could interview used their own local seeds from previous harvests. When using other

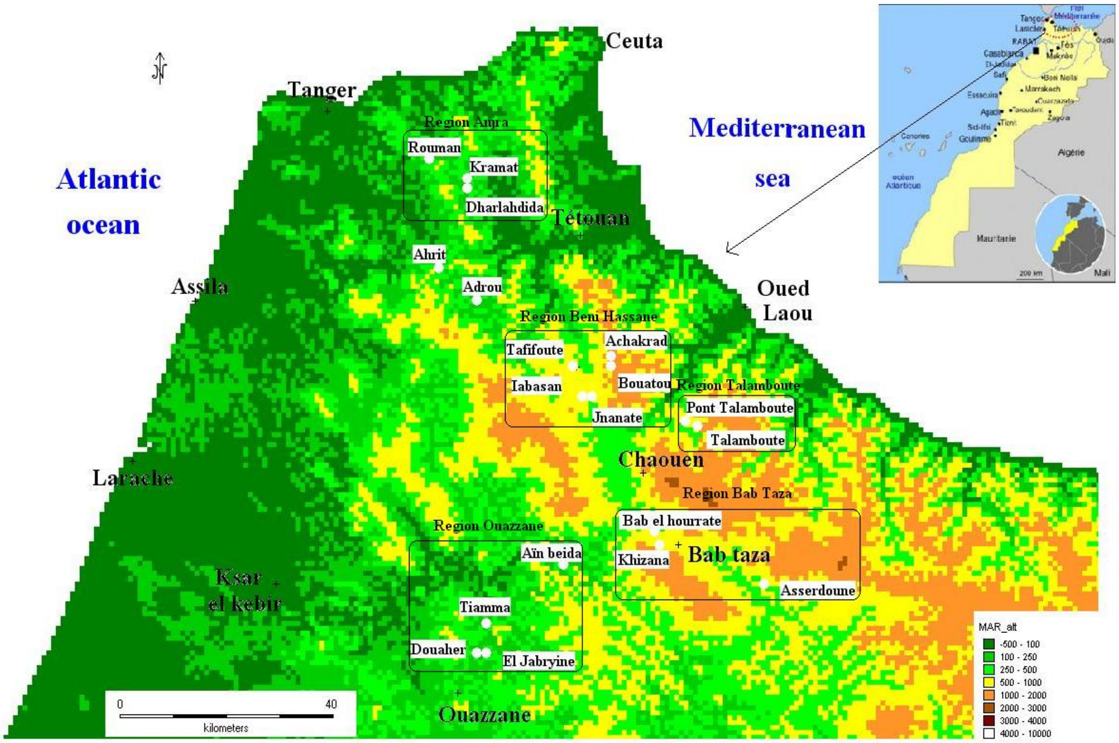

Figure 1. Location of the surveyed sites with background of altitude above sea level (edited by Diva-Gis).

Table 1. Localization of sampled populations

Region	Locality name	Longitude East	Latitude North	Altitude (m)
Anjra	Kramat	05’34,780	35’40,638	301
	Dharlahdida	05’34,307	35’39,893	373
	Rouman	05’38,098	35’32,080	175
Ahrit	Ahrit	05’33,511	35’38,990	224
Adrou	Adrou	05’33,236	35’25,308	566
Beni Hassane	Achakrad	05’29,985	35’23,729	468
	Tafifoute	05’22,355	35’23,507	647
	Bouatou	05’25,355	35’221,507	713
	Jnanate	05’23,301	35’17,876	720
	Iabasan	05’25,504	35’16,551	819
Talamboute	Talamboute	05’18,888	35’18,262	504
	Pont Talamboute	05’20,992	35’18,979	419
Bab Taza	Khizana	05’14,750	35’03,583	825
	Bab el hourrate	05’04,150	34’59,348	492
	Asserdoune	05’03,178	34’59,797	644
Ouazzane	Ain Beida	05’24,647	35’01,393	185
	Tiamma	05’32,011	34’55,654	136
	Douaher	05’33,317	34’52,120	228
	El Jabriyine	05’32,703	34’52,914	170

Table 2. List of the measured characters.

Character type	Character	Code
	Germination time (Days)	GT
Germination	Precocity (Days)	PR
	Percentage of germination (%)	PG
	Plant height (mm)	PLH
	Stem diameter (mm)	SD
	Length of the first internode (mm)	LFI
	Number of leaf per plant	NLPL
Morphology	Leaf Length (mm)	LL
	Leaf width (mm)	LW
	Pod Length (mm)	POL
	Pod width (mm)	POW
	First flowering date (Days)	FF
Phenology	Duration of flowering (Days)	F
	Days of pod maturity (Days)	FDPOM
	Maturity (Days)	M
	Total biomass (g/plant)	BY
	Seed yield (g/plant)	SY
	Harvest index (%)	HI
Productivity	Number of thallus per plant	NTPL
	Number of pods per plant	NPOPL
	Pod yield (g)	POY
	Number of seeds per pod	NSPO
	Hundred seeds weight (g)	HSW

seeds than their own, they either used seeds from other farmers in the same village (dchar) or bought some at the local "souk" (Weekly rural market). Therefore, the seeds used have a short geographical circuit. Indeed, the souk geographical range of attraction is limited (Troin, 1975) and usually which corresponds to the mountainous regions of catchment areas. We can then consider that the seeds are specific to each catchment area with a very limited circulation and exchange level in relation to materialized connections like major roads.

Seed quality

It was important to check the seed quality used by farmers. For this, we conducted germination tests. The results showed that the majority of seeds have an excellent germination capacity and are therefore of good quality. Indeed, average germination rate for all populations is about 96% with a level of variation being less than 7%. However, comparison of populations showed a significant differences among them. The lowest rate of germination was found in Asserdoune (73.33%) and Bab el hourrate (86.67%). At the opposite 9 populations showed a 100% germination rate and 8 populations ranged between 93.3 and 96.7%. With regard to the precocity (PR), the seeds of *V. ervilia* revealed precocious with rapid germination and lack of dormancy. The range of variation of precocity varied between 1 and 2 days (Table 3) with slight differences were observed between the different populations, with two (Kramat and Dharlahdida) showing a quicker germination, starting during the second day. The total time of the germination (GT) is short, varying between 2 and 3 days.

Phenology

The phenological stages observed focused on both the first flowering date and pod formation. From the agro-climatic point of view, they are interesting and major adaptive traits. Our results showed a low level of variation among populations, the results being very homogeneous and the analysis of variance not showing any significant effect (Table 4). On average it took 47 days for the appearance of the first flowers (FF) and maximum flowering (F) is reached between 48 and 50.36 days. In

Table 3. The main parameters of the seeding measure for 19 populations of *Vicia ervilia*.

Code	Mean ± SD	Min.	Max.	CV (%)
GT (Days)	2.26±0.45	2.00	3.00	19.99
PR (Days)	1.10±0.31	1.00	2.00	28.53
PG (%)	95.96±6.53	73.33	100.00	6.81

SD: Standard deviation, Min: Observed minimal value, Max : Observed maximal value, CV: Coefficient of variation.

Table 4. The parameters of the phenology measures for 19 populations of *Vicia ervilia*.

Code	Mean ± SD	Min.	Max.	CV (%)	F Pop.	F Reg.
FF (Days)	47.42±0.5	47.00	48.00	1.07		
F (Days)	48.89±0.71	48.00	50.36	1.45	1.52NS	2.41*
FDPOM (Days)	72.26±0.45	72.00	73.00	0.63		
M (Days)	73.25±0.28	72.71	73.70	0.40	1.49NS	2.79*

SD: Standard deviation, Min: Observed minimal value, Max: Observed maximal value, CV: Coefficient of variation, F Pop.: Report of variance of the pop effect, df (18). F Reg.: Report of variance of the region effect, df (6). NS : Not Significant, * Significant at p=0.095, ** Significant with p=0.090, *** Significant with p=0.099.

comparison with other studies, we can consider our populations as early flowering, and not spread out in populations of northern Morocco. Indeed, Larbi et al. (2011) measured an average of 93 days to detected the first flower and Mebarkia and Abelguerfi (2007) estimated it as 72.5 days. Regarding pod formation (FDPOM) and their maturities (M), the populations studied presented an early production with few variation among them (CV< 1%). Indeed, the first pods were observed after 72 days and the maximum of maturity was observed between 72.71 and 73.70 days. The values reported in other studies are much higher and show a later production date estimated to 141 days (Larbi et al., 2011) and 127.4 days (Mebarkia and Abelguerfi, 2007). The large difference between these values and those obtained in our study can be explained by the fact that we grow our plants in greenhouse contrary to these previous studies that have led experimental crops fields. Another important result is the low level of variation among populations, the differences observed in these parameters not exceeding 3 days. The only significant variations are among regions, while the study of Larbi et al. (2011) showed a 17 days difference among accessions to reach flowering and 40 days to pod maturity, Saxena et al. (1993) also revealing differences (14 days to flowering and 18 days to pod maturity). The difference in the level of variability is most certainly explained by the range of the genetic basis of the populations included in the study. Indeed, the previous studies used accessions from the ICARDA collection that come from a broader geographical area than our study with its narrow geographical sampling. However, the ecotypes of northern Morocco are interesting, since they are precocious and have a short cycle. This is an important character in the agro-climatic level in the Mediterranean context with erratic weather and tending towards aridity.

Morphology

To get an idea of the variability of shapes and sizes within and among populations, we studied traits related to plant (height, length of internodes, stem diameter), leaves (length, width) and pods (length, width) morphologies. The results obtained showed a variable level of variation depending on the characters (Table 5). For example, the coefficient of variation for pods characters is around 10%, without any significant variation either at the inter-population level or inter-regions. The average size of pods (18.9 mm of long to 4.7 mm of width) is similar to that observed by Berger et al. (2002). There is therefore no differentiation for this character. Conversely, width leaves showed a highly significant variation both at inter-pop and inter-regions levels. The height of the plants also showed also significant variations. The average height (PLH) measured is 525.83 mm with a variation between the populations of Bab el hourrate (457.5 mm) and those of Ain Beida (586.5 mm). This variation is larger than the 329 mm observed by Berger et al. (2002). The importance of these phenotypic variations predicts a significant genetic variation despite the geographical scale reduced, leaving open the possibility for varietal selection.

Production

The traits related to biomass production and grains are of great agronomical interest. The importance of the

Table 5. The parameters of the morphology and productivity measures for 19 populations of *Vicia ervilia*.

Code	Mean ± SD	Min.	Max.	CV (%)	F Pop.	F Reg.
PLH (mm)	525.83±31.62	457.50	586.50	6.01	2.51**	2.41*
SD (mm)	2.04±0.18	1.74	2.39	9.14	1.56NS	3.33**
LFI (mm)	33.71±7.33	22.25	58.81	21.76	2.25**	1.43NS
NLPL	13.18±1.03	11.20	14.70	7.86	1.31NS	2.05NS
LL (mm)	106.19±23.44	91.52	200.57	22.08	1.32NS	2.92*
LW (mm)	18.9±2.2	15.54	22.48	11.65	2.99***	5.82 ***
POL (mm)	18.92±1.9	14.83	21.18	10.08	1.63NS	1.29 NS
POW (mm)	4.77±0.39	4.03	5.26	8.23	1.41NS	1.75NS
BY (g/plant)	4.52±0.33	3.84	5.23	7.33	1.81*	3.58**
SY (g/plant)	0.34±0.11	0.13	0.60	34.48	1.29NS	2.31*
HI (%)	6.16±2.46	2.50	10.62	39.95	1.41NS	2.711*
NTPL	2.51±0.19	2.10	2.80	7.87	1.17NS	0.88NS
NPOPL	4.81±1.3	2.10	6.70	27.06	1.24NS	1.55NS
POY (g)	0.07±0.02	0.03	0.13	31.03	1.64NS	2.76*
NSPO	2.92±0.29	2.37	3.50	10.10	1.35NS	1.76NS
HSW (g)	3.92±0.44	3.30	5.09	11.32	-	-

SD: Standard deviation, Min: Observed minimal value, Max: Observed maximal value, CV: Coefficient of variation, F Pop.: Report of variance of the pop effect, df (18). F Reg.: Report of variance of the region effect, df (6). NS: Not Significant, * Significant with p=0,095, ** Significant with p=0,090, *** Significant with p=0,099.

variation is dependent on the character studied (Table 5), ranging from 39.9% for the harvest index (HI) to only 7.33% for the biomass (BY). The biomass estimated by the weight of the plants (BY) is the only character showing a significant population effect. Conversely, at the among-region level, the differentiation seems to be more important and we observed significant variations for 4 characters: biomass (BY), seed production (SY), harvest index (HI) and the weight of the pod (POY). Differentiation between the different ecotypes appears to be distance-related and due (at least partially) to the isolation between crop areas. For the harvest index (HI), the values varied between 2.50% (Asserdoune) and 10.62% (Rouman) with a mean of 6.16% and a high coefficient of variation (39.95%). Compared to other available data, this estimation appears to be relatively high compared to those given by Berger et al. (2002) and Larbi et al. (2011) and less than that the one obtained by Saxena et al. (1993). This difference could be explained by the difference in experimental devices and the way to calculate the HI. Regarding the weight of the pod (POY), the mean value is 0.07 g with a CV of 31.03%. Although the population effect is not significant, the difference observed between some populations is important, for example between Bab el hourrate (0.03 g) and Achakrad (0.13 g).

Structuring variability

In order to explain the variance in the data using all measured parameters, we conducted a principal component analysis (PCA). This analysis was performed with a matrix encompassing the 19 surveyed populations of *V. ervilia* and 23 characters considered in the study. An important part of the information is correlated with the first two factorial axes containing 44.58% of the total variance (Table 6). Variance expressed by the axis 1 (31.70%) is mainly due to the strong correlation (Table 6) between traits related to production, such as size and weight of pods and seeds. Axis 2 (12.88%) is rather correlated with characters related to phenology and morphology. The population projection in the factorial plan (1, 2) (Figure 2) show a structuring gradient along axis 1 with a negative side isolating the 3 populations sampled in the region of Bab Taza. The remaining populations clustered in an opposite pole, forming a gradient along axis 2, the populations of Anjra (north) to that of Ouazzane (south) and an intermediate position with other populations (Beni Hassane and Talamboute). This gradient structured populations according to reproductive parameters, such as phenology, morphology and germination. This structure has a geographical meaning, corresponding to a differentiation related to both distance and physical isolation. Indeed, the region of Bab Taza belongs to an isolated area with relatively few connections with other regions, thus limiting possibility of exchanges. Other populations belong to more connected regions, where seeds trade would be more easily among them. The fact that it is a mountainous area with rugged terrain may explain the differentiation on such a small scale.

Conclusions

From a genetic resources management perspective view,

Table 6. Eigen values and contribution of characters to the variance of axis.

Parameter	F1	F2
Eigen values	7.292	2.962
Variance (%)	31.706	12.877
Cumulate variance (%)	31.706	44.583
Contribution of characters (%)		
BY	1.508	12.085
SY	9.237	0.175
HI	6.334	4.310
NTPL	1.532	2.963
NPOPL	4.229	0.030
POL	8.544	0.472
POW	9.977	0.172
POY	9.259	1.329
NSPO	3.082	2.749
HSW	6.083	0.254
FF	4.484	4.333
F	5.734	2.191
FDPOM	0.012	10.386
M	0.108	24.607
PLH	0.774	4.321
SD	1.019	16.083
LFI	2.328	1.103
NLPL	5.126	0.883
LL	1.147	0.472
LW	2.835	7.287
GT	6.980	0.856
PR	1.981	1.411
PG	7.688	1.527

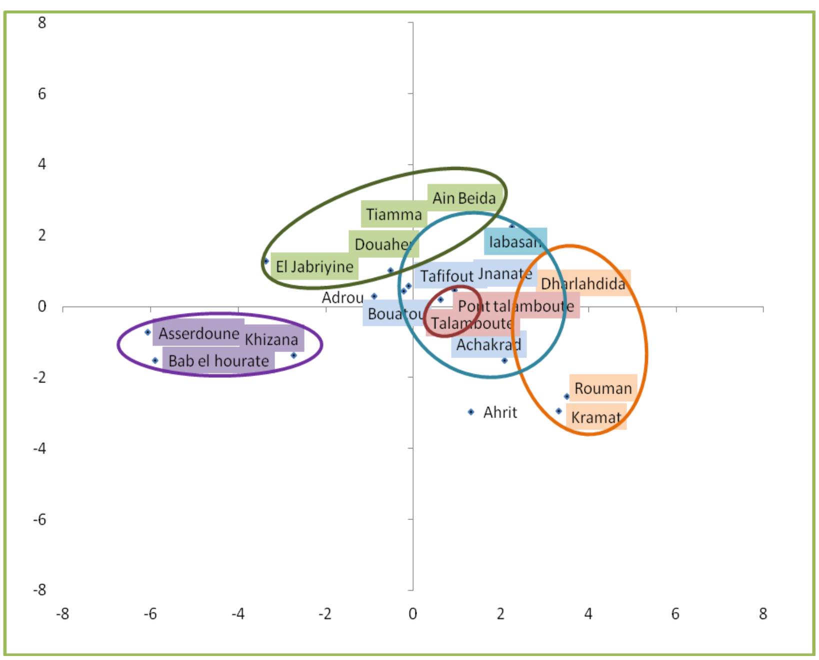

Figure 2. Projection on the plan (1.2) of the principal component analysis (CPA).

this study confirmed the existence of local populations of *V. ervilia,* maintained by farmers in a traditional agriculture frame. The evaluation of the diversity of these populations with a set of characters (germination, phenology, morphology and production) showed the existence of significant variability for several characters, with differentiation between regions representing the various production areas. At a phenotypic level, we showed a significant degree of variability which suggests the existence of significant genetic diversity within and among these populations. An approach using genetic markers will enable us to validate this hypothesis and measure the genetic diversity encompassed within our sampling. At the agronomic level, and in relation with the adaptive potential of these populations in regards to tolerance to drought and high temperature, the populations we studied showed interesting skills for selection. In particular, the lack of dormancy, the germination capacity, the precocity of flowering, pod maturity and a short life cycle are important characters to be selected in this context of global warming and climate change.

From a yield point, surveyed populations present an interesting potential both as a forage crop biomass and grain production. The size and weight of seeds is similar to those observed in other Mediterranean areas. The harvest index is relatively high and shows that these populations are more adapted for grain productions. Traits related to production show some variability and differentiating between regions. However, it would be advisable to check these capabilities by field trials.

Conflict of Interests

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

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Abbreviation: GRIN, Germplasm Resources Information Network; **IBPGR,** International Board for Plant Genetic Resources; **FAO,** Food and Agriculture Organization; **IPCC,** intergovernmental panel on climate change; **ICARDA,** International Centre for Agricultural Research in Dry Areas.

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Rural development through the inclusion of family farming into the biodiesel production chain: Learning from Brazilian experience

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This article presents a critical review of the Brazilian government's initiative to use biodiesel production to promote rural development by means of the inclusion of family farmers in the biodiesel production chain. The paper describes the dynamics of the National Program for Production and Use of Biodiesel (PNPB) and comments some of its results. Both the number of farmers included and their income have been increasing from year to year. However, more than a half of the contracts of PNPB are being made with family farmers from the south of the country, who are more organized and tecnified than in other regions of Brazil. Although there have been advances, the government still faces difficulties to promote regional development based on biodiesel production in the poorest regions, e.g, the North and Northeast. The entrance of Petrobras (Public Limited Company of Oil and Bioenergy), changes in the regulatory framework of this sector and PNPB can help policies promote regional development and revitalize the social aspect of the program.

Key words: Biodiesel, competitiveness, family famers, social inclusion, National Program for Production and Use of Biodiesel (PNPB).

INTRODUCTION

Some factors are stimulating countries to pursue alternatives to fossil fuels. There are predictions about reduction of oil production in traditional areas, the expensive costs of drilling in newly discovered reserves elsewhere around the globe, the consciousness about harm the consumption of non-renewable fuels brings, the instability of global supply and related geopolitical issues (César, 2009). According to data retrieved by International Energy Agency, in 2030, biofuels will replace between 4 and 7% of all fossil fuel energy consumption worldwide (IEA, 2004b). In Brazil the instability situation in supply conditions may be mitigated

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by recent oil discoveries in its coastal waters (Freire, 2008). However, the undesired effect of the increased use of fuels in the country remains a challenge to be faced. Due to the limited potential for biofuels production in Europe and USA and lower production costs in Latin American countries, these developed countries are expected to meet a fraction of their mandatory blending targets through imported biofuels. This can create market opportunities for biofuels production in Brazil (Janssen and Rutz, 2011).

Brazil has significant competitive advantages in the overall production of biofuels, especially in biodiesel. Moreover, renewable energy sources account for 44% of the Brazilian energy matrix, three times more than the world energy matrix (IEA, 2004). Another important Brazilian advantage in biofuel production is the availability of agricultural areas that can be used for agro-energy activities, which is not a reality in most countries (Frondel and Peter, 2007). An example of this is that Brazil was the pioneer in the production of ethanol fuel for transport (Perosa, 2012) and sugarcane bagasse to generate steam and electricity (César and Batalha, 2009).

Policies encouraging the production and use of biofuels have been implemented in major consumer countries worldwide. The explicit support for large scale production of liquid biofuels suggests that energy security is a far more important motivation (Horst and Vermeylen, 2011). This was also the driver for the first Brazilian program for bioenergy production from biomass, mainly for the production of ethanol fuel (Moreira and Goldemberg, 1999).

The National Program for Production and Use of Biodiesel (or in Brazil, just PNPB – Programa Nacional de Produção e Uso do Biodiesel) subverts this logic, stimulating the biodiesel production as a tool to promote sustainable rural development and to mitigate poverty in the field (Perosa, 2012).

The PNPB instituted the mandatory addition of biodiesel to petroleum diesel as well as a set of policies to encourage the diversification of the Brazilian energy matrix (César and Batalha, 2009; Pousa et al., 2007; Garcez and Vianna, 2009).

The most important action of PNPB was establishing the Law that guarantees a minimum addition of biodiesel to diesel fuel produced in Brazilian territory, thus creating demand. In 2004, the blend of 2% biodiesel (in volume) to diesel oil (that is, B2) was instituted in the country on a voluntary basis. This first blend became mandatory in 2008. In July 2008, B3 was adopted as a compulsory biodiesel blend, which was increased to 4% in July 2009 and then to 5% in January 2011, which is currently upheld (César and Batalha, 2010).

Brazil is a huge producer of biofuel. The country produced 2.7 billion liters in 2012. Additionally, in July 2012, the sector totaled 64 biodiesel plants authorized by the Brazilian National Agency of Petroleum, Natural Gas and Biofuels (ANP) to operate in the country, corresponding to an approved total capacity of 19,533.95 m^3/day, of which 61 already have approval for commercialization the biodiesel produced (a capacity of 18,606.25 m^3/day) (ANP, 2013b).

An important difference of PNPB compared to the initiatives of other countries is that the Brazilian government also sees it as a rural development strategy, which is based on the insertion of family farmers into the biodiesel production chain. In fact, it can even be said that the social inclusion was a priority of PNPB. Unquestionably, the intention of PNPB is a fair policy for a large part of Brazil's poor rural community to reach a new era of biofuel production in the country (Carioca et al., 2009). However, the support for large scale production resulted in the program being criticized.

In order to focus efforts for the inclusion of family farmers into PNPB, the Social Fuel Seal (SFS) was created (Pousa et al., 2007; Garcez and Vianna, 2009; César and Batalha, 2010; Watanabe et al., 2012). Basically, the SFS provides agreements between small farmers and biodiesel mills. This certification is formulated for companies that prove the purchase of the raw material used in the biodiesel industry. The amount is determined by the Ministry of Agrarian Development and varies according to the region of the country (César, 2009; César and Batalha, 2010).

One issue that makes PNPB successful is the volume of biodiesel produced, but many other objectives of the Program were not achieved. One of them was the diversification of raw materials sources for biodiesel production. Currently, soybean oil supplies approximately 80% of the biodiesel produced in Brazil (ANP, 2013a).

Another incomplete objective is the inclusion of family farmers in this production system. The initial goal was to include 200 thousand families in PNPB. In total, there were 104,295 family farming operations established in 2011, proportionally distributed, 58.5% in the South, 35.7% in Northeast and 5.8% are located in other regions of the country. Despite the increasing participation of farmers from the Northeast over the past few years, they received only 0.5% of the total payments of PNPB in 2011 for family farmers, which reflects the structural deficiencies of family farming in the region (Brazilian Ministry of Agrarian Development, 2012).

High transactions costs involved in managing a large number of family farmers have been an obstacle to the PNPB initiative. Furthermore, despite the real and potential growth of the Brazilian biodiesel market, its production is still surrounded by many doubts, particularly concerning its long-term sustainability and economic viability.

Some experiments done with jatropha, palm and cottonseed cultivated making use of irrigation systems and technical assistance in San Francisco Valley (the Semi-arid Region of Northeast of Brazil) have shown that the integration between family farmers and biodiesel mills, with social inclusion, rural development, and economic-financial feasibility is possible (Sanches et al., 2009; Lima Jr. et al., 2008).

In this sense, this paper presents a critical review of Brazil's initiative to use biodiesel production as a way to promote rural development based on family farming. It describes the dynamics of these initiatives and presents some of the results of these actions.

This article presents the methodological procedures to elaborate the article. This is followed by considerations on the relevance to continue promoting the biodiesel industry. The importance of social inclusion policies in Brazil with regards to biodiesel production was then discussed followed by a presentation of PNPB, its efforts to advance the inclusion of family farmers and some of the results achieved. Finallly, the final considerations are presented.

METHODOLOGICAL PROCEDURES

The article can be classified as a theoretical essay. The methodological procedures were limited to Desk Research and Documental Analysis. These methods have been selected because any scientific article should have a desk research to build its theoretical basis, and a documental analysis as a basis for primary data collection.

The Desk Research is a mode of study and analysis of scientific documents, such as books, journals, theoretical essays and other scientific papers. It is, therefore, a study of scientific sources that deal with the subject under research. The Documental Analysis, in turn, characterizes itself by finding information in documents not received any scientific treatment as executive reports, articles from newspapers, magazines, letters, films, recordings, photographs, and other promotional materials (Oliveira, 2007).

This article considers the Desk Research and Documental Analysis as integrated and complementary methods of research, that is, both secondary and primary data are of vital importance for the development of the work.

THE LACK OF COST COMPETITIVENESS FOR BIODIESEL PRODUCTION

From the economic viewpoint, competitiveness can be interpreted as the ability of an organization, sector, region or country to sustainably support market competition (Charlier, 2001). However, changes in consumer behavior, production standards and valuation of social and environmental aspects by society and the market, have led to increased competitive analysis dimensions.

Worldwide, the production costs of biofuels are not competitive in comparison to those derived from fossil raw materials (Hass and Foglia, 2006; Wassel and Dittmer, 2006; Duer and Christensen, 2009). However, there are, some exceptions, such as diesel obtained from residual oils and Brazilian sugarcane ethanol (Perosa, 2012; Peters and Thielmann, 2008; Timilsina and Shrestha, 2011; Peters and Thielmann, 2008).

The differential of productivity explains the competitiveness of Brazilian sugarcane ethanol. Brazil produces an average 6,800 L of sugarcane ethanol per hectare, while the EU produces 5,400 L/ha of beet ethanol and only 2,400 L/ha of wheat ethanol, and the US 3,100 L/ha of corn ethanol. This fact helps Brazil to produce the one of the cheapest ethanol in the world (Licht's, 2007).

Although high oil prices and decrease in production costs through learning curve make biofuels more competitive, they still need policy support measures to compete with gasoline and diesel from fossil sources (Timilsina and Shrestha, 2011). Biofuel cost reductions will depend on the cumulative experience in emerging technologies that could overcome this economic barrier (Lensink and Londo, 2009).

In the case of Brazilian sugarcane based ethanol, an indicator called progress ratio (PR) is used to describe the learning curve. For example, a PR of 80% means that the cost declines 20% for each doubling of production. The lower the PR the faster the decline in cost. Data for the cost of ethanol indicates that in an initial phase the ethanol prices did not decline very rapidly (PR of approximately 80%), but in a more mature phase decreased very rapidly (PR of 50%) followed by a period of stagnation (PR of 90%) (Moreira and Goldemberg, 1999).

In 2010, the Department of Planning and Investment (DPI) of the Ministry of Planning concluded that "in the recent past and near future, the production of biodiesel is not economically viable" (César, 2012). Anyway, the economic aspects are just one of the pillars of the federal program. Concerning the other pillars – social and environmental –, there was no evaluation of the mechanisms of integration in the sector of the biodiesel production chain with family farmers, not even the environmental advances for biofuels (ONG Repórter Brasil, 2010a).

Thus, considering only the aspects related to economic issues to justify the competitive operation of a system is a questionable practice (César, 2009; Perosa, 2012). The Brazilian ethanol production is a good example of this new way of defining a competitive production system. Although the production of ethanol fuel has one of the best economic cost benefit ratio available today, there is much criticism regarding the social and environmental impacts that may have been generated by its production in Brazil (Perosa, 2012). Deforestation of the Amazon forest, degradation of the Cerrado Biome (Brazilian Savanna), environmental and social impacts of sugarcane harvest, displacement of indigenous communities and food–fuel conflicts are also mentioned in the literature as unwanted impacts (Janssen and Rutz, 2011).

Because of this, the ethanol sector in Brazil is trying to defend the competitiveness based on its positive energy and GHG balance more than cost efficiency. The energy balance (renewable energy in the biofuel divided by fossil energy used to produce it) depends on the feedstock for ethanol: corn in the US (1.4), sugar cane in Brazil (8.3), wheat and beet in Europe (2). The same analysis for biodiesel shows: oil palm (9), residues of vegetable oils (5.5), soybean (3) and colza (2.5) (WWI, 2006). On the other hand, ethanol from sugar cane (Brazil) contributes with about 85% of the GHG reduction, ethanol from grains (US and EU) 30%, and ethanol from beet (EU) 45%. In comparison to diesel, biodiesel reduces approximately in 50% the volume of CO2 emitted (IEA, 2004).

Climate change awareness has been an important additional driver to stimulate biofuels given that it helps to mitigate climate change by displacing fossil fuel consumption (Timilsina and Shrestha, 2011). In line with this concept, the lack of cost competitiveness could be counterbalanced by the reduction of greenhouse gas emissions (Duer and Christensen, 2009).

As most biofuels exported to Europe from Latin America are expected to fall under the criteria of sustainability, producers and trading countries will have to engage in systems that monitor and verify this fulfillment with sustainability requirements (Janssen and Rutz, 2011).

Soybean sector in Brazil, for example, is looking for RTRS (Roundtable on Responsible Soybean) certification as a way to proof the compliance with sustainability demand. However, there are some barriers like: i) informational asymmetry (about environmental and social laws, good agricultural practices etc.); ii) high costs of compliance (3 years average payback for a small and medium farmers; 1 year average payback for big farmers); iii) lack of financial incentives (weak demand for certification as consumer countries like China does not need environmental protocols) (KPMG, 2013).

The social and environmental benefits and the lack of economical competitiveness can explain the reason why biofuels programs were created under the protection of mandatory blending targets and specific regulations or grating subsidies through tax waivers (Peters and Thielmann, 2008, Knothe 2006b, Charles et al., 2007).

Generally, for biofuel to be competitive, it is necessary some governmental incentives to reduce the learning cost, especially in its initial phase. Many developed countries have established national policies aimed at increasing the domestic production of biofuels (Horst and Vermeylen, 2011). One of these is Brazil. The mandatory blending targets associated with PNPB created a strong domestic demand for biodiesel (César, 2009). Correspondingly, this law strives to use this demand to stimulate the production of oilseeds for the production of biodiesel by family farmers, in order to ensure their inclusion in this productive chain.

THE IMPORTANCE OF PROMOTING SOCIAL INCLUSION (INCLUSION OF FAMILY FAMERS)

It should be emphasized that the definition of “family farming” is not a consensus. Typically, the classification of an agricultural activity known as “family farmer” involves technical, economic and social criteria. The profile of family farming is considered as all-inclusive and is associated with the historical formation of groups, the cultural heritage, the professional and personal experience and the access and availability of a different set of factors, which include natural resources, human capital and social capital (Buainain, 2007).

The image of the family farmer is often mistakenly associated with that of a simple countryman, naïve and backward, with a sense of subsistence production (Altafin, 2007). But, family production is a flexible category that goes from subsistence agriculture to highly technified monoculture (Wilkinson, 1997). In other words, family farming comprises a range of families, such as those making a living in smallholdings and in extreme poverty as well as those inserted in modern agribusiness structures (Buainain, 2005).

However, the main concept of family farming used in this article is related to the eligibility criteria to access the National Program for Strengthening Family Farming (or in Brazil, just PRONAF). This program entitles family farmer to obtain credit at low interest rates (Watanabe et al., 2012). In order to get access to credit with low rates, the farmer needs to have PRONAF’s Aptitude Declaration, that is, the DAP (Brazilian Federal Senate, 2010).

In general, PRONAF considers family farming as one activity with all of the following characteristics: i) the family lives in the rural property; ii) the property area cannot exceed four fiscal modules (unit of measure that varies according to the region of Brazil); iii) the activities in the rural property should be responsible from 30% until 70% of family income; iv) the family labor needs to be the main labor force in the property; v) the annual family income cannot exceed US$ 55 thousand (Brazilian Federal Senate, 2010).

The literature reports that family farmers unable to adapt to the competitive environment are naturally excluded from the market. However, family farmers have a hard time competing with the production of the major international agricultural commodities where scale economies are decisive to the success of the undertaking. The advantages of family farming are increasingly associated with a differentiated agriculture – niche, organic and/or artisan (Wilkinson, 2007).

In the same direction, even for the biofuel sector, consumers in developed countries could provide social benefits by paying a premium price for socially and environmentally certified biofuels imported from developing countries (Horst and Vermeylen, 2011).

However, experiences in Germany and in the UK – where government subsidies are essential to the competitiveness of biodiesel – have already demonstrated that consumers will only use biodiesel and ethanol if their prices are more attractive than the petroleum-derived diesel and gasoline fuels (Bomb et al, 2007).

It is good to emphasize that consumer preferences may have changed since, and concerns for sustainability may have grown to an extent of calling into question the assumption that price would still been decisive for this product.

Conventionally, studies that analyze the economics of vegetable oil production have focused on large-scale biodiesel production facilities, although there is a growing literature dealing with the economics of small-scale biofuel production (Schumacher, 2007). Whether the benefits associated with the biodiesel based on vegetable oils are maintained or enhanced through a reduction in scale remains unclear (Fore et al., 2011).

The social impacts of biofuel production may have difficulties because of the pressure to increase the production of liquid biofuels, considered as global commodities. In this sense, the economic logic that drives investment decisions in commercial companies clearly favors large-scale monoculture plantations to maximize yields in the short term. This production model (that is, imposed by a powerful stakeholder – in this case, biodiesel mills) is particularly poor in delivery of non-market goods and services to local communities or future generations, which lack the power to avoid that type of development or negotiate a fair compensation (Horst and Vermeylen, 2011).

Consequently, the differences in agricultural schemes, the family farming sector at one hand and the agro-industrial sector on the other hand, should be considered. Particularly, the social impacts of biofuel production are related to the conflicting goals between these two sectors (Janssen and Rutz, 2011).

The exclusion of family farmers from the production of commodities also results in “social diseconomies” to be paid by the whole society. The migration of rural workers to the cities, and the government that may have to bear the costs of helping them, can be an example of such “diseconomies”. Furthermore, it is important to emphasize the social and psychological problems of such workers, as a result of the loss of their original community identity (Foladori, 1999).

Higher levels of production concentration and specialization are among the factors leading to this exclusion, which, in turn, also implies in the social costs that are inherent to the disarticulation of the diverse family farming structure (Wilkinson, 1997). Thus, it is important to recognize the importance of agribusiness a socially more economical way to maintain the rural population in the field. These policies could be justified even if they represent higher production costs for certain products (Foladori, 1999).

The most vulnerable producers would be the priority targets of social policies in order to support their production activities. Empowering these farmers would be justified more by the need to avoid the social debt with family farming than for reasons related to increasing their production competitiveness in the field.

Family farming, when strengthened, has the ability to boost the economy that surrounds it. By generating purchasing power, it also generates jobs in local businesses, and therefore jobs in the supply chains, which in turn results in increased local incomes. Thus, the development with income distribution in the rural sector brings also the development of the urban sector (Pedroso, 2000).

Brazil has been at the forefront with proposals for rural development. On the one hand, State is indeed the only politically legitimate actor to propose, implement and enforce deliberate mechanisms towards social change. The State becomes the primary agent to implement specific government actions, with the main intention of developing the rural areas of the country (Navarro, 2001).

Accordingly, with respect to the Brazilian policies regarding the social inclusion and the biodiesel sector, the institution of Law 11.097/2005 stands out, which not only established the compulsory

Table 1. Increase in the number of family agriculture organizations participating in PNPB by region, from 2005 to 2011.

Brazilian Region	2005	2006	2007	2008	2009	2010	2011
South	-	8,736	27,928	8,767	29,150	52,187	60,993
Northeast	15,000	30,226	6,850	17,187	17,711	41,253	37,226
Center West	-	1,441	1,690	1,662	2,550	3,388	3,533
Southeast	914	7	55	27	1,457	3,297	2,486
North	414	185	223	215	179	246	57
Total	16,328	40,595	36,746	27,858	51,047	100,371	104,295

Source: Elaborated from Brazilian Ministry of Agrarian Development (2011, 2012).

addition of biodiesel to diesel fuel for consumers, anywhere in the country, but which also determined measures to encourage the social inclusion of family farmers, that is, those with DAP (Brazilian Federal Government, 2005).

The goals of social inclusion embedded in PNPB are very much discussed in Brazil. These discussions reflect the current limitations of the models to evaluate competitiveness. An analysis that disregards the financial or monetary metrics to assess the competitiveness of a production system, and which underlies its assessment on the impacts of this system into the society where it is inserted, may consider that any financial disadvantages could be compensated by environmental and social gains for the whole of this society.

PNPB: THE DYNAMICS OF THE SOCIAL FUEL SEAL AND ITS RESULTS

The milestone of PNPB was the determination of Law 11.097/2005, which established the mandatory addition of 2% biodiesel to diesel fuel at the end of the commercial chain. The sale of biodiesel happens through auctions of the National Agency of Petroleum, Natural Gas and Biofuels (NAP). Another priority of such public purchases is to foster the integration among biodiesel producers and family farmers, that is, the auctions require companies to have the Social Fuel Seal (SFS) for thetransactions in this market.To obtain the SFS, the biodiesel company must sign technical cooperation and trade agreements with the cooperatives of small producers, or directly with the family farmers. SFS brings tax advantages (tax exemption), allows access to the 80% of the ANP auctions, promotes better financing conditions from public banks and serves as a positive marketing tool for companies that have it.

After obtaining the certification, the company must purchase a minimum percentage of raw materials from family farmers. This amount is defined by the Ministry of Agrarian Development (MDA) and it is differentiated according to the region in which the company is located. According to the currently rules (that is, normative No. 01/2009), the minimum percentage for the acquisition of raw material from biodiesel family farmers to obtain the SFS is 15% for the acquisitions from the north and mid-west regions, and 30% for the acquisitions from the south, southeast, northeast, and semi-arid regions (Brazilian Federal Government, 2009).

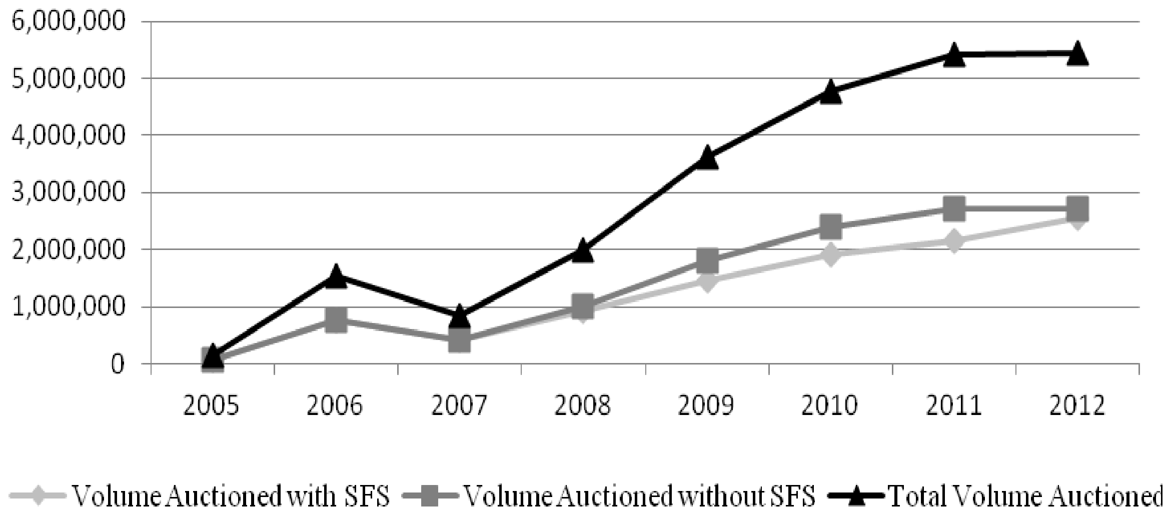
The SFS was the main instrument created by PNPB to ensure the social inclusion (César, 2009, 2012; Garcez and Vianna, 2009; Navarro, 2001; César and Batalha, 2011). Graph 1 shows the volumes auctioned in ANP auctions since 2005. In 2012, 2.7 billion liters of biodiesel were auctioned, of which 2.5 billion liters were supplied by companies compulsorily having the SFS (ANP, 2013b).

The government had initially planned to include 200 thousand PNPB families. Table 1 shows the growth in the number of family farmers benefited by the inclusion policy in recent years. However, the regions of the Midwest, Southeast and North have contributed little to social inclusion via PNPB (Brazilian Ministry of Agrarian Development, 2011, 2012).

The Brazilian government has been facing difficulties to achieve the goals proposed at the beginning of PNPB (César, 2009, 2012; César and Batalha, 2010). In 2006, for instance, more than 30 thousand farmers were contracted in the Northeast of Brazil. However, the following year this number decreased substantially in 2007 (Table 1) (Brazilian Ministry of Agrarian Development, 2011, 2012).

In some cases, the tax exemption inherent to the SFS did not compensate the efforts of the companies producing biodiesel. In this situation, the private investments were insufficient to ensure the proper functioning of the production arrangements in the Northeast of the country. The non-compliance of the terms of agreements between companies and farmers resulted in the farmers' loss of trust in the companies as well as in PNPB (César and Batalha, 2013). A major barrier to PNPB's social goals is the structural weaknesses of family farming that along with other minor reasons account for not reaching the objectives.

Some studies report the enormous difficulties faced by companies in achieving the agreements with family farmers producing castor oil, mainly in northeastern Brazil (César, 2009; César and Batalha, 2010, 2013; Watanabe et al., 2012; Wilkinson and Herrera, 2010). These are some of the difficulties in this region: low production rate; the geographical spread of the assisted families; the technological restrictions of the product process; the



Graph 1. Volume de biodiesel auctioned (in billions of liters) from biodiesel mills with/without SCS and its total during the period of 2005 to 2012. Source: Elaborated from ANP (2013b).

low productivity; the inadequate handling; the highly irregular seasonality; the inefficient technical assistance; the great influence of intermediaries on the castor oil chain; the unstable prices; the inexperienced associations; the high debts of farmers, and; the difficulties in obtaining credit.

In the North of the country, the projects with palm are pilots and are taken as a reference for the inclusion of family farmers in the national biodiesel production chain. However, some difficulties are also reported in these cases (César, 2012; César and Batalha, 2013). The palm projects grow more slowly due to the high investments necessary to implement this cultivation, since, in most cases plantations are combined with the processing of the palm clusters. The long period between seed germination and the first harvest make the return on investment distant. Bureaucratic restrictions (such as seed purchases and conflicting landowner issues) and the lack of infrastructure (not only roads, but social issues such as schools, leisure etc.) render hard the companies' production process (César, 2012; César and Batalha, 2013).

The soybean family farming for biodiesel production is concentrated in the south of the country. Theseproducers are more technical-oriented than the castor and palm family farmers. Moreover, most of these farmers provide their crops to organized cooperatives or negotiate directly with the biodiesel production industry.

In Brazil soybean has competitive advantages against other oilseed crops. The level of technological development attained allows soybean to be produced in several regions of the country. The efficient management of these family farmers who assemble in cooperatives to manage the demands imposed by PNPB should be emphasized. It makes the soybean from the families farmers disputed by the companies in the sector. The risk of breach of contract and the need for compliance with the quotas acquired by the family farmers meant that companies were willing to pay a premium for the so-called "social soybean", which varies between contracts, reaching up to US$0.75/bag (César, 2012).

The social agreements for the production of biodiesel from raw materials originating in family farming are still concentrated in the South, which is where there is 52% of the total farmers that provide the raw material to SFS biodiesel companies. Those farmers are crucial for the existence of the SFS, since they account for about 94% of the biodiesel produced in the PNPB (Table 2) (Brazilian Ministry of Agrarian Development, 2011, 2012). It should be mentioned that the involvement of rural communities in the biofuel sector is complex and it cannot be evaluated through simplistic proxies such as the number of jobs on the plantation or the average wage per worker. It requires more detailed analysis of how the livelihood strategies and outcomes of rural communities and the individuals within these are transformed by the changes in land ownership, land administration and land use associated with the shift to biofuel production (Horst and Vermeylen, 2011).

Productive inclusion projects of farmers are mostly developed in communities with strong social demands. It is therefore vital that technical assistance actions for these farmers should be accompanied by activities to improve these populations' social capital and overall health conditions, safety, housing and schooling. Due to the precarious conditions where these projects are developed, it would be necessary to not only provide technical assistance to the communities, but also minimal training – such as education and basic ideas of hygiene – to enable these people foresee the perspective of a better life (Abreu et al., 2009).

Farmers are assisted and strongly stimulated to create

Table 2. Supply acquired from family farmers by PNPB from 2008 to 2011.

Feedstock (%)	2008	2009	2010	2011
Soy bean	92.59	94.60	94.06	98.28
Castor bean	1.86	3.96	4.38	0.51
Soy Oil	3.69	0.65	0.51	0.91
Sesame	0.00	0.03	0.39	0.02
Palm	0.89	0.37	0.32	0.00
Sunflower	0.71	0.17	0.11	0.11
Canola	0.22	0.05	0.11	0.13
Peanut	0.04	0.18	0.10	0.05
Others	0.01	0.03	-	-
Total	**100**	**100**	**100**	**100**

Source: Elaborated from Brazilian Ministry of Agrarian Development (2011, 2012).

'Family Farmer Cooperatives', acting as the intermediary between the smallholders and the biodiesel producers (Janssen and Rutz, 2011). However, poor family farmers are not aware of the importance of these actions and therefore fail to understand the culture of collaboration. Additionally, the image of cooperatives in the Northeast is relatively negative. Family farmers do not trust in these organizations due to political influence and corruption in their decision making processes and administration (Abreu et al., 2009).

This scenario undermines these families' endeavor for the efficient and effective functioning of the projects promoted by PNPB. However, it should be emphasized that efforts have been directed to overcome these difficulties. From the standpoint of inclusive goals, the results of the program are still vulnerable, but it cannot be concluded that the social arrangements are a failure.

Despite the marginalization of family farming in the beginning of the program, the effort to include these farmers in the biodiesel production chain is acknowledged. Currently, the scenario looks a little different, since the number of family farmers grew from 40,595 to 104,295 (Brazilian Ministry of Agrarian Development, 2011).

The ingress of Petrobras (the Brazilian Governmental Oil and Bioenergy Company) in the sector, as well as the changes in the former normative, contributed significantly to promote the PNPB. The outcome of the efforts by Petrobras will also be crucial to the success of the social pillars of the PNPB (La Rovere et al., 2011).

FINAL REMARKS

Brazil stands apart from other countries as it uses PNPB as a rural development strategy through the integration of family farmers into the biodiesel production chain. The research depicted in this article holds the view that the Brazilian government did not fully achieve the initial goals designed by this federal program. Particularly, the goal of economic insertion of family farmers throughout the remote and difficult to access regions into the biodiesel production chain is far from being achieved.

It is acknowledged that there have been advances. The number of families integrated in the biodiesel productive chains has increased each year, as well as the income of the family farmers involved in these production systems.

Notwithstanding, the main goal of PNPB is not being reached, because more than half of the PNPB agreements are carried out with family farmers from the south of the country, primarily soybean producers. These farmers have a good production structure, cooperative tradition and a more favorable socioeconomic status than those of the North and Northeast of the country. The government is facing difficulties to promote regional development in the notoriously penniless regions of the country. This situation, as explained earlier, is in conflict with the real objectives of PNPB, which is strongly guided by the social development of poor areas.

The prolonged process of economic marginalization that characterizes much of Brazil's family farmers calls not only for productive inclusion jobs, but also for actions that lead to in-depth cultural changes in these communities. All these changes are important to increase the social capital of these farmers and consequently encourage and increase the effectiveness of their collective actions.

Even so, it is important to emphasize that efforts have been driven to overcome these difficulties. For now, the entrance of Petrobras into the biodiesel production, as well as the changes in the former normative, contributed significantly to revitalize the social pillar of the program. Because of it, this new chapter of PNPB deserves to be closely monitored. As suggestion for future studies, we recommend to evaluate in detail successful cases of integration between small farmers and biodiesel mills as well as failure cases. This process is good to understand the better contractual arrangements according to different regions and oilseeds.

Conflict of Interests

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

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Full Length Research Paper

Rural development through the inclusion of family farming into the biodiesel production chain: Learning from Brazilian experience

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This article presents a critical review of the Brazilian government's initiative to use biodiesel production to promote rural development by means of the inclusion of family farmers in the biodiesel production chain. The paper describes the dynamics of the National Program for Production and Use of Biodiesel (PNPB) and comments some of its results. Both the number of farmers included and their income have been increasing from year to year. However, more than a half of the contracts of PNPB are being made with family farmers from the south of the country, who are more organized and tecnified than in other regions of Brazil. Although there have been advances, the government still faces difficulties to promote regional development based on biodiesel production in the poorest regions, e.g, the North and Northeast. The entrance of Petrobras (Public Limited Company of Oil and Bioenergy), changes in the regulatory framework of this sector and PNPB can help policies promote regional development and revitalize the social aspect of the program.

Key words: Biodiesel, competitiveness, family famers, social inclusion, National Program for Production and Use of Biodiesel (PNPB).

INTRODUCTION

Some factors are stimulating countries to pursue alternatives to fossil fuels. There are predictions about reduction of oil production in traditional areas, the expensive costs of drilling in newly discovered reserves elsewhere around the globe, the consciousness about harm the consumption of non-renewable fuels brings, the instability of global supply and related geopolitical issues (César, 2009). According to data retrieved by International Energy Agency, in 2030, biofuels will replace between 4 and 7% of all fossil fuel energy consumption worldwide (IEA, 2004b). In Brazil the instability situation in supply conditions may be mitigated

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by recent oil discoveries in its coastal waters (Freire, 2008). However, the undesired effect of the increased use of fuels in the country remains a challenge to be faced. Due to the limited potential for biofuels production in Europe and USA and lower production costs in Latin American countries, these developed countries are expected to meet a fraction of their mandatory blending targets through imported biofuels. This can create market opportunities for biofuels production in Brazil (Janssen and Rutz, 2011).

Brazil has significant competitive advantages in the overall production of biofuels, especially in biodiesel. Moreover, renewable energy sources account for 44% of the Brazilian energy matrix, three times more than the world energy matrix (IEA, 2004). Another important Brazilian advantage in biofuel production is the availability of agricultural areas that can be used for agro-energy activities, which is not a reality in most countries (Frondel and Peter, 2007). An example of this is that Brazil was the pioneer in the production of ethanol fuel for transport (Perosa, 2012) and sugarcane bagasse to generate steam and electricity (César and Batalha, 2009).

Policies encouraging the production and use of biofuels have been implemented in major consumer countries worldwide. The explicit support for large scale production of liquid biofuels suggests that energy security is a far more important motivation (Horst and Vermeylen, 2011). This was also the driver for the first Brazilian program for bioenergy production from biomass, mainly for the production of ethanol fuel (Moreira and Goldemberg, 1999).

The National Program for Production and Use of Biodiesel (or in Brazil, just PNPB – Programa Nacional de Produção e Uso do Biodiesel) subverts this logic, stimulating the biodiesel production as a tool to promote sustainable rural development and to mitigate poverty in the field (Perosa, 2012).

The PNPB instituted the mandatory addition of biodiesel to petroleum diesel as well as a set of policies to encourage the diversification of the Brazilian energy matrix (César and Batalha, 2009; Pousa et al., 2007; Garcez and Vianna, 2009).

The most important action of PNPB was establishing the Law that guarantees a minimum addition of biodiesel to diesel fuel produced in Brazilian territory, thus creating demand. In 2004, the blend of 2% biodiesel (in volume) to diesel oil (that is, B2) was instituted in the country on a voluntary basis. This first blend became mandatory in 2008. In July 2008, B3 was adopted as a compulsory biodiesel blend, which was increased to 4% in July 2009 and then to 5% in January 2011, which is currently upheld (César and Batalha, 2010).

Brazil is a huge producer of biofuel. The country produced 2.7 billion liters in 2012. Additionally, in July 2012, the sector totaled 64 biodiesel plants authorized by the Brazilian National Agency of Petroleum, Natural Gas and Biofuels (ANP) to operate in the country, corresponding to an approved total capacity of 19,533.95 m^3/day, of which 61 already have approval for commercialization the biodiesel produced (a capacity of 18,606.25 m^3/day) (ANP, 2013b).

An important difference of PNPB compared to the initiatives of other countries is that the Brazilian government also sees it as a rural development strategy, which is based on the insertion of family farmers into the biodiesel production chain. In fact, it can even be said that the social inclusion was a priority of PNPB. Unquestionably, the intention of PNPB is a fair policy for a large part of Brazil's poor rural community to reach a new era of biofuel production in the country (Carioca et al., 2009). However, the support for large scale production resulted in the program being criticized.

In order to focus efforts for the inclusion of family farmers into PNPB, the Social Fuel Seal (SFS) was created (Pousa et al., 2007; Garcez and Vianna, 2009; César and Batalha, 2010; Watanabe et al., 2012). Basically, the SFS provides agreements between small farmers and biodiesel mills. This certification is formulated for companies that prove the purchase of the raw material used in the biodiesel industry. The amount is determined by the Ministry of Agrarian Development and varies according to the region of the country (César, 2009; César and Batalha, 2010).

One issue that makes PNPB successful is the volume of biodiesel produced, but many other objectives of the Program were not achieved. One of them was the diversification of raw materials sources for biodiesel production. Currently, soybean oil supplies approximately 80% of the biodiesel produced in Brazil (ANP, 2013a).

Another incomplete objective is the inclusion of family farmers in this production system. The initial goal was to include 200 thousand families in PNPB. In total, there were 104,295 family farming operations established in 2011, proportionally distributed, 58.5% in the South, 35.7% in Northeast and 5.8% are located in other regions of the country. Despite the increasing participation of farmers from the Northeast over the past few years, they received only 0.5% of the total payments of PNPB in 2011 for family farmers, which reflects the structural deficiencies of family farming in the region (Brazilian Ministry of Agrarian Development, 2012).

High transactions costs involved in managing a large number of family farmers have been an obstacle to the PNPB initiative. Furthermore, despite the real and potential growth of the Brazilian biodiesel market, its production is still surrounded by many doubts, particularly concerning its long-term sustainability and economic viability.

Some experiments done with jatropha, palm and cottonseed cultivated making use of irrigation systems and technical assistance in San Francisco Valley (the Semi-arid Region of Northeast of Brazil) have shown that the integration between family farmers and biodiesel mills, with social inclusion, rural development, and economic-financial feasibility is possible (Sanches et al., 2009; Lima Jr. et al., 2008).

In this sense, this paper presents a critical review of Brazil's initiative to use biodiesel production as a way to promote rural development based on family farming. It describes the dynamics of these initiatives and presents some of the results of these actions.

This article presents the methodological procedures to elaborate the article. This is followed by considerations on the relevance to continue promoting the biodiesel industry. The importance of social inclusion policies in Brazil with regards to biodiesel production was then discussed followed by a presentation of PNPB, its efforts to advance the inclusion of family farmers and some of the results achieved. Finallly, the final considerations are presented.

METHODOLOGICAL PROCEDURES

The article can be classified as a theoretical essay. The methodological procedures were limited to Desk Research and Documental Analysis. These methods have been selected because any scientific article should have a desk research to build its theoretical basis, and a documental analysis as a basis for primary data collection.

The Desk Research is a mode of study and analysis of scientific documents, such as books, journals, theoretical essays and other scientific papers. It is, therefore, a study of scientific sources that deal with the subject under research. The Documental Analysis, in turn, characterizes itself by finding information in documents not received any scientific treatment as executive reports, articles from newspapers, magazines, letters, films, recordings, photographs, and other promotional materials (Oliveira, 2007).

This article considers the Desk Research and Documental Analysis as integrated and complementary methods of research, that is, both secondary and primary data are of vital importance for the development of the work.

THE LACK OF COST COMPETITIVENESS FOR BIODIESEL PRODUCTION

From the economic viewpoint, competitiveness can be interpreted as the ability of an organization, sector, region or country to sustainably support market competition (Charlier, 2001). However, changes in consumer behavior, production standards and valuation of social and environmental aspects by society and the market, have led to increased competitive analysis dimensions.

Worldwide, the production costs of biofuels are not competitive in comparison to those derived from fossil raw materials (Hass and Foglia, 2006; Wassel and Dittmer, 2006; Duer and Christensen, 2009). However, there are, some exceptions, such as diesel obtained from residual oils and Brazilian sugarcane ethanol (Perosa, 2012; Peters and Thielmann, 2008; Timilsina and Shrestha, 2011; Peters and Thielmann, 2008).

The differential of productivity explains the competitiveness of Brazilian sugarcane ethanol. Brazil produces an average 6,800 L of sugarcane ethanol per hectare, while the EU produces 5,400 L/ha of beet ethanol and only 2,400 L/ha of wheat ethanol, and the US 3,100 L/ha of corn ethanol. This fact helps Brazil to produce the one of the cheapest ethanol in the world (Licht's, 2007).

Although high oil prices and decrease in production costs through learning curve make biofuels more competitive, they still need policy support measures to compete with gasoline and diesel from fossil sources (Timilsina and Shrestha, 2011). Biofuel cost reductions will depend on the cumulative experience in emerging technologies that could overcome this economic barrier (Lensink and Londo, 2009).

In the case of Brazilian sugarcane based ethanol, an indicator called progress ratio (PR) is used to describe the learning curve. For example, a PR of 80% means that the cost declines 20% for each doubling of production. The lower the PR the faster the decline in cost. Data for the cost of ethanol indicates that in an initial phase the ethanol prices did not decline very rapidly (PR of approximately 80%), but in a more mature phase decreased very rapidly (PR of 50%) followed by a period of stagnation (PR of 90%) (Moreira and Goldemberg, 1999).

In 2010, the Department of Planning and Investment (DPI) of the Ministry of Planning concluded that "in the recent past and near future, the production of biodiesel is not economically viable" (César, 2012). Anyway, the economic aspects are just one of the pillars of the federal program. Concerning the other pillars – social and environmental –, there was no evaluation of the mechanisms of integration in the sector of the biodiesel production chain with family farmers, not even the environmental advances for biofuels (ONG Repórter Brasil, 2010a).

Thus, considering only the aspects related to economic issues to justify the competitive operation of a system is a questionable practice (César, 2009; Perosa, 2012). The Brazilian ethanol production is a good example of this new way of defining a competitive production system. Although the production of ethanol fuel has one of the best economic cost benefit ratio available today, there is much criticism regarding the social and environmental impacts that may have been generated by its production in Brazil (Perosa, 2012). Deforestation of the Amazon forest, degradation of the Cerrado Biome (Brazilian Savanna), environmental and social impacts of sugarcane harvest, displacement of indigenous communities and food–fuel conflicts are also mentioned in the literature as unwanted impacts (Janssen and Rutz, 2011).

Because of this, the ethanol sector in Brazil is trying to defend the competitiveness based on its positive energy and GHG balance more than cost efficiency. The energy balance (renewable energy in the biofuel divided by fossil energy used to produce it) depends on the feedstock for ethanol: corn in the US (1.4), sugar cane in Brazil (8.3), wheat and beet in Europe (2). The same analysis for biodiesel shows: oil palm (9), residues of vegetable oils (5.5), soybean (3) and colza (2.5) (WWI, 2006). On the other hand, ethanol from sugar cane (Brazil) contributes with about 85% of the GHG reduction, ethanol from grains (US and EU) 30%, and ethanol from beet (EU) 45%. In comparison to diesel, biodiesel reduces approximately in 50% the volume of CO2 emitted (IEA, 2004).

Climate change awareness has been an important additional driver to stimulate biofuels given that it helps to mitigate climate change by displacing fossil fuel consumption (Timilsina and Shrestha, 2011). In line with this concept, the lack of cost competitiveness could be counterbalanced by the reduction of greenhouse gas emissions (Duer and Christensen, 2009).

As most biofuels exported to Europe from Latin America are expected to fall under the criteria of sustainability, producers and trading countries will have to engage in systems that monitor and verify this fulfillment with sustainability requirements (Janssen and Rutz, 2011).

Soybean sector in Brazil, for example, is looking for RTRS (Roundtable on Responsible Soybean) certification as a way to proof the compliance with sustainability demand. However, there are some barriers like: i) informational asymmetry (about environmental and social laws, good agricultural practices etc.); ii) high costs of compliance (3 years average payback for a small and medium farmers; 1 year average payback for big farmers); iii) lack of financial incentives (weak demand for certification as consumer countries like China does not need environmental protocols) (KPMG, 2013).

The social and environmental benefits and the lack of economical competitiveness can explain the reason why biofuels programs were created under the protection of mandatory blending targets and specific regulations or grating subsidies through tax waivers (Peters and Thielmann, 2008, Knothe 2006b, Charles et al., 2007).

Generally, for biofuel to be competitive, it is necessary some governmental incentives to reduce the learning cost, especially in its initial phase. Many developed countries have established national policies aimed at increasing the domestic production of biofuels (Horst and Vermeylen, 2011). One of these is Brazil. The mandatory blending targets associated with PNPB created a strong domestic demand for biodiesel (César, 2009). Correspondingly, this law strives to use this demand to stimulate the production of oilseeds for the production of biodiesel by family farmers, in order to ensure their inclusion in this productive chain.

THE IMPORTANCE OF PROMOTING SOCIAL INCLUSION (INCLUSION OF FAMILY FAMERS)

It should be emphasized that the definition of "family farming" is not a consensus. Typically, the classification of an agricultural activity known as "family farmer" involves technical, economic and social criteria. The profile of family farming is considered as all-inclusive and is associated with the historical formation of groups, the cultural heritage, the professional and personal experience and the access and availability of a different set of factors, which include natural resources, human capital and social capital (Buainain, 2007).

The image of the family farmer is often mistakenly associated with that of a simple countryman, naïve and backward, with a sense of subsistence production (Altafin, 2007). But, family production is a flexible category that goes from subsistence agriculture to highly technified monoculture (Wilkinson, 1997). In other words, family farming comprises a range of families, such as those making a living in smallholdings and in extreme poverty as well as those inserted in modern agribusiness structures (Buainain, 2005).

However, the main concept of family farming used in this article is related to the eligibility criteria to access the National Program for Strengthening Family Farming (or in Brazil, just PRONAF). This program entitles family farmer to obtain credit at low interest rates (Watanabe et al., 2012). In order to get access to credit with low rates, the farmer needs to have PRONAF's Aptitude Declaration, that is, the DAP (Brazilian Federal Senate, 2010).

In general, PRONAF considers family farming as one activity with all of the following characteristics: i) the family lives in the rural property; ii) the property area cannot exceed four fiscal modules (unit of measure that varies according to the region of Brazil); iii) the activities in the rural property should be responsible from 30% until 70% of family income; iv) the family labor needs to be the main labor force in the property; v) the annual family income cannot exceed US$ 55 thousand (Brazilian Federal Senate, 2010).

The literature reports that family farmers unable to adapt to the competitive environment are naturally excluded from the market. However, family farmers have a hard time competing with the production of the major international agricultural commodities where scale economies are decisive to the success of the undertaking. The advantages of family farming are increasingly associated with a differentiated agriculture – niche, organic and/or artisan (Wilkinson, 2007).

In the same direction, even for the biofuel sector, consumers in developed countries could provide social benefits by paying a premium price for socially and environmentally certified biofuels imported from developing countries (Horst and Vermeylen, 2011).

However, experiences in Germany and in the UK – where government subsidies are essential to the competitiveness of biodiesel – have already demonstrated that consumers will only use biodiesel and ethanol if their prices are more attractive than the petroleum-derived diesel and gasoline fuels (Bomb et al, 2007).

It is good to emphasize that consumer preferences may have changed since, and concerns for sustainability may have grown to an extent of calling into question the assumption that price would still been decisive for this product.

Conventionally, studies that analyze the economics of vegetable oil production have focused on large-scale biodiesel production facilities, although there is a growing literature dealing with the economics of small-scale biofuel production (Schumacher, 2007). Whether the benefits associated with the biodiesel based on vegetable oils are maintained or enhanced through a reduction in scale remains unclear (Fore et al., 2011).

The social impacts of biofuel production may have difficulties because of the pressure to increase the production of liquid biofuels, considered as global commodities. In this sense, the economic logic that drives investment decisions in commercial companies clearly favors large-scale monoculture plantations to maximize yields in the short term. This production model (that is, imposed by a powerful stakeholder – in this case, biodiesel mills) is particularly poor in delivery of non-market goods and services to local communities or future generations, which lack the power to avoid that type of development or negotiate a fair compensation (Horst and Vermeylen, 2011).

Consequently, the differences in agricultural schemes, the family farming sector at one hand and the agro-industrial sector on the other hand, should be considered. Particularly, the social impacts of biofuel production are related to the conflicting goals between these two sectors (Janssen and Rutz, 2011).

The exclusion of family farmers from the production of commodities also results in "social diseconomies" to be paid by the whole society. The migration of rural workers to the cities, and the government that may have to bear the costs of helping them, can be an example of such "diseconomies". Furthermore, it is important to emphasize the social and psychological problems of such workers, as a result of the loss of their original community identity (Foladori, 1999).

Higher levels of production concentration and specialization are among the factors leading to this exclusion, which, in turn, also implies in the social costs that are inherent to the disarticulation of the diverse family farming structure (Wilkinson, 1997). Thus, it is important to recognize the importance of agribusiness a socially more economical way to maintain the rural population in the field. These policies could be justified even if they represent higher production costs for certain products (Foladori, 1999).

The most vulnerable producers would be the priority targets of social policies in order to support their production activities. Empowering these farmers would be justified more by the need to avoid the social debt with family farming than for reasons related to increasing their production competitiveness in the field.

Family farming, when strengthened, has the ability to boost the economy that surrounds it. By generating purchasing power, it also generates jobs in local businesses, and therefore jobs in the supply chains, which in turn results in increased local incomes. Thus, the development with income distribution in the rural sector brings also the development of the urban sector (Pedroso, 2000).

Brazil has been at the forefront with proposals for rural development. On the one hand, State is indeed the only politically legitimate actor to propose, implement and enforce deliberate mechanisms towards social change. The State becomes the primary agent to implement specific government actions, with the main intention of developing the rural areas of the country (Navarro, 2001).

Accordingly, with respect to the Brazilian policies regarding the social inclusion and the biodiesel sector, the institution of Law 11.097/2005 stands out, which not only established the compulsory

Table 1. Increase in the number of family agriculture organizations participating in PNPB by region, from 2005 to 2011.

Brazilian Region	2005	2006	2007	2008	2009	2010	2011
South	-	8,736	27,928	8,767	29,150	52,187	60,993
Northeast	15,000	30,226	6,850	17,187	17,711	41,253	37,226
Center West	-	1,441	1,690	1,662	2,550	3,388	3,533
Southeast	914	7	55	27	1,457	3,297	2,486
North	414	185	223	215	179	246	57
Total	16,328	40,595	36,746	27,858	51,047	100,371	104,295

Source: Elaborated from Brazilian Ministry of Agrarian Development (2011, 2012).

addition of biodiesel to diesel fuel for consumers, anywhere in the country, but which also determined measures to encourage the social inclusion of family farmers, that is, those with DAP (Brazilian Federal Government, 2005).

The goals of social inclusion embedded in PNPB are very much discussed in Brazil. These discussions reflect the current limitations of the models to evaluate competitiveness. An analysis that disregards the financial or monetary metrics to assess the competitiveness of a production system, and which underlies its assessment on the impacts of this system into the society where it is inserted, may consider that any financial disadvantages could be compensated by environmental and social gains for the whole of this society.

PNPB: THE DYNAMICS OF THE SOCIAL FUEL SEAL AND ITS RESULTS

The milestone of PNPB was the determination of Law 11.097/2005, which established the mandatory addition of 2% biodiesel to diesel fuel at the end of the commercial chain. The sale of biodiesel happens through auctions of the National Agency of Petroleum, Natural Gas and Biofuels (NAP). Another priority of such public purchases is to foster the integration among biodiesel producers and family farmers, that is, the auctions require companies to have the Social Fuel Seal (SFS) for thetransactions in this market.To obtain the SFS, the biodiesel company must sign technical cooperation and trade agreements with the cooperatives of small producers, or directly with the family farmers. SFS brings tax advantages (tax exemption), allows access to the 80% of the ANP auctions, promotes better financing conditions from public banks and serves as a positive marketing tool for companies that have it.

After obtaining the certification, the company must purchase a minimum percentage of raw materials from family farmers. This amount is defined by the Ministry of Agrarian Development (MDA) and it is differentiated according to the region in which the company is located. According to the currently rules (that is, normative No. 01/2009), the minimum percentage for the acquisition of raw material from biodiesel family farmers to obtain the SFS is 15% for the acquisitions from the north and mid-west regions, and 30% for the acquisitions from the south, southeast, northeast, and semi-arid regions (Brazilian Federal Government, 2009).

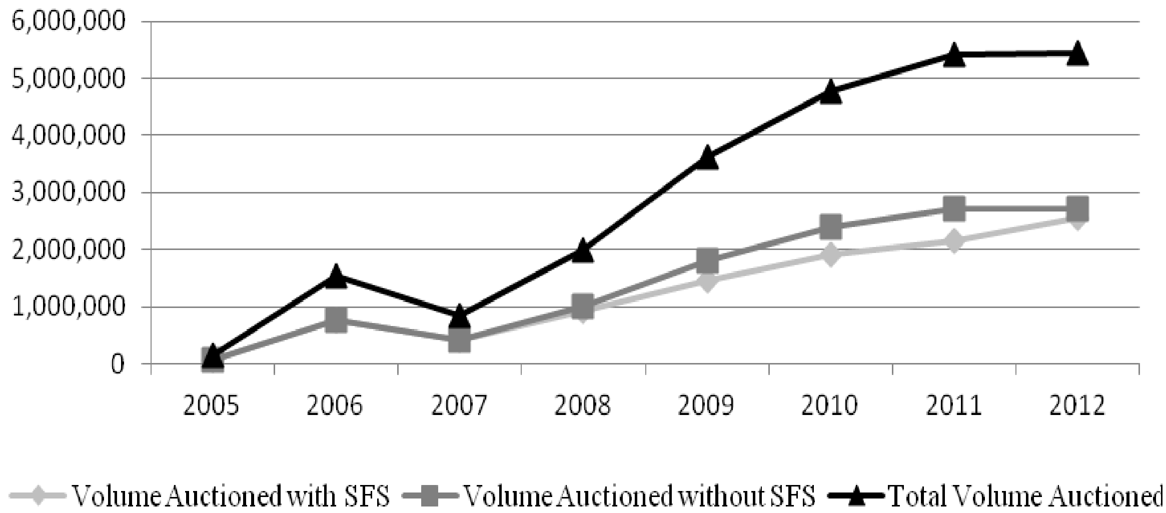
The SFS was the main instrument created by PNPB to ensure the social inclusion (César, 2009, 2012; Garcez and Vianna, 2009; Navarro, 2001; César and Batalha, 2011). Graph 1 shows the volumes auctioned in ANP auctions since 2005. In 2012, 2.7 billion liters of biodiesel were auctioned, of which 2.5 billion liters were supplied by companies compulsorily having the SFS (ANP, 2013b).

The government had initially planned to include 200 thousand PNPB families. Table 1 shows the growth in the number of family farmers benefited by the inclusion policy in recent years. However, the regions of the Midwest, Southeast and North have contributed little to social inclusion via PNPB (Brazilian Ministry of Agrarian Development, 2011, 2012).

The Brazilian government has been facing difficulties to achieve the goals proposed at the beginning of PNPB (César, 2009, 2012; César and Batalha, 2010). In 2006, for instance, more than 30 thousand farmers were contracted in the Northeast of Brazil. However, the following year this number decreased substantially in 2007 (Table 1) (Brazilian Ministry of Agrarian Development, 2011, 2012).

In some cases, the tax exemption inherent to the SFS did not compensate the efforts of the companies producing biodiesel. In this situation, the private investments were insufficient to ensure the proper functioning of the production arrangements in the Northeast of the country. The non-compliance of the terms of agreements between companies and farmers resulted in the farmers' loss of trust in the companies as well as in PNPB (César and Batalha, 2013). A major barrier to PNPB's social goals is the structural weaknesses of family farming that along with other minor reasons account for not reaching the objectives.

Some studies report the enormous difficulties faced by companies in achieving the agreements with family farmers producing castor oil, mainly in northeastern Brazil (César, 2009; César and Batalha, 2010, 2013; Watanabe et al., 2012; Wilkinson and Herrera, 2010). These are some of the difficulties in this region: low production rate; the geographical spread of the assisted families; the technological restrictions of the product process; the



Graph 1. Volume de biodiesel auctioned (in billions of liters) from biodiesel mills with/without SCS and its total during the period of 2005 to 2012. Source: Elaborated from ANP (2013b).

low productivity; the inadequate handling; the highly irregular seasonality; the inefficient technical assistance; the great influence of intermediaries on the castor oil chain; the unstable prices; the inexperienced associations; the high debts of farmers, and; the difficulties in obtaining credit.

In the North of the country, the projects with palm are pilots and are taken as a reference for the inclusion of family farmers in the national biodiesel production chain. However, some difficulties are also reported in these cases (César, 2012; César and Batalha, 2013). The palm projects grow more slowly due to the high investments necessary to implement this cultivation, since, in most cases plantations are combined with the processing of the palm clusters. The long period between seed germination and the first harvest make the return on investment distant. Bureaucratic restrictions (such as seed purchases and conflicting landowner issues) and the lack of infrastructure (not only roads, but social issues such as schools, leisure etc.) render hard the companies' production process (César, 2012; César and Batalha, 2013).

The soybean family farming for biodiesel production is concentrated in the south of the country. Theseproducers are more technical-oriented than the castor and palm family farmers. Moreover, most of these farmers provide their crops to organized cooperatives or negotiate directly with the biodiesel production industry.

In Brazil soybean has competitive advantages against other oilseed crops. The level of technological development attained allows soybean to be produced in several regions of the country. The efficient management of these family farmers who assemble in cooperatives to manage the demands imposed by PNPB should be emphasized. It makes the soybean from the families farmers disputed by the companies in the sector. The risk of breach of contract and the need for compliance with the quotas acquired by the family farmers meant that companies were willing to pay a premium for the so-called "social soybean", which varies between contracts, reaching up to US$0.75/bag (César, 2012).

The social agreements for the production of biodiesel from raw materials originating in family farming are still concentrated in the South, which is where there is 52% of the total farmers that provide the raw material to SFS biodiesel companies. Those farmers are crucial for the existence of the SFS, since they account for about 94% of the biodiesel produced in the PNPB (Table 2) (Brazilian Ministry of Agrarian Development, 2011, 2012). It should be mentioned that the involvement of rural communities in the biofuel sector is complex and it cannot be evaluated through simplistic proxies such as the number of jobs on the plantation or the average wage per worker. It requires more detailed analysis of how the livelihood strategies and outcomes of rural communities and the individuals within these are transformed by the changes in land ownership, land administration and land use associated with the shift to biofuel production (Horst and Vermeylen, 2011).

Productive inclusion projects of farmers are mostly developed in communities with strong social demands. It is therefore vital that technical assistance actions for these farmers should be accompanied by activities to improve these populations' social capital and overall health conditions, safety, housing and schooling. Due to the precarious conditions where these projects are developed, it would be necessary to not only provide technical assistance to the communities, but also minimal training – such as education and basic ideas of hygiene – to enable these people foresee the perspective of a better life (Abreu et al., 2009).

Farmers are assisted and strongly stimulated to create

Table 2. Supply acquired from family farmers by PNPB from 2008 to 2011.

Feedstock (%)	2008	2009	2010	2011
Soy bean	92.59	94.60	94.06	98.28
Castor bean	1.86	3.96	4.38	0.51
Soy Oil	3.69	0.65	0.51	0.91
Sesame	0.00	0.03	0.39	0.02
Palm	0.89	0.37	0.32	0.00
Sunflower	0.71	0.17	0.11	0.11
Canola	0.22	0.05	0.11	0.13
Peanut	0.04	0.18	0.10	0.05
Others	0.01	0.03	-	-
Total	**100**	**100**	**100**	**100**

Source: Elaborated from Brazilian Ministry of Agrarian Development (2011, 2012).

'Family Farmer Cooperatives', acting as the intermediary between the smallholders and the biodiesel producers (Janssen and Rutz, 2011). However, poor family farmers are not aware of the importance of these actions and therefore fail to understand the culture of collaboration. Additionally, the image of cooperatives in the Northeast is relatively negative. Family farmers do not trust in these organizations due to political influence and corruption in their decision making processes and administration (Abreu et al., 2009).

This scenario undermines these families' endeavor for the efficient and effective functioning of the projects promoted by PNPB. However, it should be emphasized that efforts have been directed to overcome these difficulties. From the standpoint of inclusive goals, the results of the program are still vulnerable, but it cannot be concluded that the social arrangements are a failure.

Despite the marginalization of family farming in the beginning of the program, the effort to include these farmers in the biodiesel production chain is acknowledged. Currently, the scenario looks a little different, since the number of family farmers grew from 40,595 to 104,295 (Brazilian Ministry of Agrarian Development, 2011).

The ingress of Petrobras (the Brazilian Governmental Oil and Bioenergy Company) in the sector, as well as the changes in the former normative, contributed significantly to promote the PNPB. The outcome of the efforts by Petrobras will also be crucial to the success of the social pillars of the PNPB (La Rovere et al., 2011).

FINAL REMARKS

Brazil stands apart from other countries as it uses PNPB as a rural development strategy through the integration of family farmers into the biodiesel production chain. The research depicted in this article holds the view that the Brazilian government did not fully achieve the initial goals designed by this federal program. Particularly, the goal of economic insertion of family farmers throughout the remote and difficult to access regions into the biodiesel production chain is far from being achieved.

It is acknowledged that there have been advances. The number of families integrated in the biodiesel productive chains has increased each year, as well as the income of the family farmers involved in these production systems.

Notwithstanding, the main goal of PNPB is not being reached, because more than half of the PNPB agreements are carried out with family farmers from the south of the country, primarily soybean producers. These farmers have a good production structure, cooperative tradition and a more favorable socioeconomic status than those of the North and Northeast of the country. The government is facing difficulties to promote regional development in the notoriously penniless regions of the country. This situation, as explained earlier, is in conflict with the real objectives of PNPB, which is strongly guided by the social development of poor areas.

The prolonged process of economic marginalization that characterizes much of Brazil's family farmers calls not only for productive inclusion jobs, but also for actions that lead to in-depth cultural changes in these communities. All these changes are important to increase the social capital of these farmers and consequently encourage and increase the effectiveness of their collective actions.

Even so, it is important to emphasize that efforts have been driven to overcome these difficulties. For now, the entrance of Petrobras into the biodiesel production, as well as the changes in the former normative, contributed significantly to revitalize the social pillar of the program. Because of it, this new chapter of PNPB deserves to be closely monitored. As suggestion for future studies, we recommend to evaluate in detail successful cases of integration between small farmers and biodiesel mills as well as failure cases. This process is good to understand the better contractual arrangements according to different regions and oilseeds.

Conflict of Interests

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

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