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African Journal of Agricultural Research

Full Length Research Paper

Influence of organic substrates on growth and nutrient contents of jatobá (*Hymenaea stigonocarpa*)

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The use of native forests for the purpose of agricultural and livestock occupation have caused the degradation of native ecosystems of caatinga and cerrado. The negative impact of these activities can be minimized with the revegetation of degraded areas. With that comes the need to produce seedlings of native forest species of quality. The objective of this study was to evaluate how the addition of bagana of carnaúba, compost, and cattle manure to Quartzarenic Neosol soil affects the percentage and rate of emergence, growth, and macronutrient contents of *Hymenaea stigonocarpa* Mart (Jatobá) seedlings. The treatments consisted of the following proportions (v/v) of bagana of carnaúba, compost, and cattle manure to the soil: 0:100, 20:80, 40:60, 60:40, 80:20, and 100:0, being in a factorial (3:6), with 10 replications, and standard chemical fertilizers were used as the control. The addition of bagana of carnaúba increased height, ratio of height to diameter, aerial part dry weight, root dry weight, total dry weight, ratio of aerial part height to aerial part dry weight, and Dickson quality index of *H. stigonocarpa* seedlings, in the estimated proportion of 48:52 (bagana of carnaúba: soil), which is the most suitable substrate composition for the cultivation of this species. The substrate containing compost enabled a greater accumulation of N, P, and Ca in the shoots of the jatoba seedlings, due to increased availability of these nutrients in the substrate containing organic compost.

Key words: Bagana of carnauba, compost organic, cattle manure, macronutrients.

INTRODUCTION

The disorderly exploitation of native forests in Brazil for agriculture and livestock farming has caused disturbances in the ecosystems of Cerrado and Caatinga. To reduce the negative impact of these activities,

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introduction of strategies such as the recovery of degraded areas have been emphasized. In this context, the demand for seedlings of native tree species to restore the degraded areas has increased (Saidellis et al., 2009), mainly in the state of Piauí. Here, a significant increase in agricultural activities in Cerrado has been observed in recent years, with intensive substitution of native vegetation by cultivated crops, mainly *Glycine max* and *Zea mays*.

Obtaining quality seedlings for reforestation is essential for the recuperation of degraded areas. The development of seedlings is influenced by the substrate and several other materials that can be used alone or in combination. Traditionally, the subsoil is a widely used substrate in the region, because of the low technological level and socioeconomic status of farmers; however, it is deficient in nutrients and contains toxic levels of aluminum (Nóbrega et al., 2008). Hence, the use of substrates made from wastes of animal and/or plant origin has become an alternative for increasing the nutrient content; they also have the advantages of low cost and easy acquisition. Addition of organic matter sources contributes not only to the supply of nutrients but also to the physical characteristics of substrates, mainly to increase the water-holding capacity of soil (Caldeira et al., 2008).

Carvalho Filho et al. (2003) evaluated the production of seedlings of jatobá (*Hymenaea courbaril*) in different environments, containers, and substrate compositions, and they found that the best results were obtained using substrates containing soil, sand, and cattle manure in the ratio of 1:2:1. The authors affirm that adding cattle manure not only increases the supply of nutrients, but also improves soil fertility, aeration conditions, and water availability. Costa et al. (2005) combined vermiculite with bagana of carnaúba, a straw-like waste product generated by the extraction of wax from the film of powder that coats the leaves of the carnauba wax palm (*Copernicia cerifera* Miller). They found that substrates were formed with greater ease by the removal of soursop (*Annona muricata* L.) rootstock from the containers, probably because of the good capacity of aggregates obtained by the combination of bagana and vermiculite and the appropriate moisture retention of the components.

Hymenaea stigonocarpa Mart. ex. Hayne (Fabaceae-Caesalpinioideae), commonly known as the jatobá of Cerrado and found in the southwestern State of Piauí, Brazil, is an ornamental hermaphrodite tree that grows up to 10 m in height. The farinaceous pulp of the jatobá fruit is much sought after by many animal species that disperse the seeds, making the plant very useful for reforestation in degraded areas and restoration of arboreal vegetation (Lorenzi, 1998). To the best of our knowledge, there are no studies on the nutrition of this species when grown on organic substrates, although there are studies on the nutrient content of the foliage of native species grown on substrates containing organic compounds. Therefore, studies aimed at analyzing the seedling tissue could contribute significantly to the production system of this species.

The objective of this study was to evaluate how the addition of cattle manure, bagana of carnaúba, and organic compost to Quartzarenic Neosol soil affects the percentage and rate of emergence, growth, and macronutrient content of *H. stigonocarpa*.

MATERIALS AND METHODS

The experiment was conducted under greenhouse conditions by using a screen with 50% shade at the Universidade Federal do Piauí, Bom Jesus city, State of Piauí, Brazil (09°04′28″S and 44°21′ 31″W, with a mean altitude of 277 m).

For the composition of the substrates, we used three organic wastes—bagana of carnaúba (BC), which consists of compost produced from a mixture of tree pruning residues (mainly (fig (*Ficus* sp.), Acacia (*Acacia* sp)., *Licania tomentosa*, and *Cenostigma macrophyllum*); Cocos nucifera husk fiber (OC); and cattle manure (CM)—together with Quartzarenic Neosol soil samples sieved through a 2-mm mesh sieve with the following chemical and physical characteristics: pH (H_2O): 6.30; Soil organic matter: 6.0 g kg^{-1}; P (Mehlich): 5.10 mg dm^{-3}; K^+: 1.3 $mmol_c$ dm^{-3}; Ca^{2+}: 11.0 $mmol_c$ dm^{-3}; Mg^{2+}: 4.0 $mmol_c$ dm^{-3}; Al^{3+}: 1.0 $mmol_c$ dm^{-3}; H+Al: 18.0 $mmol_c$ dm^{-3}; Base sum: 16.3 $mmol_c$ dm^{-3}; Cation exchange capacity: 34.3 $mmol_c$ dm^{-3}; Base saturation: 48.0%; Al^{3+} saturation: 6.1%, B: 0.19 mg dm^{-3}, Cu^{2+}: 0.20 mg dm^{-3}; Fe^{2+}: 15.0 mg dm^{-3}:Mn^{2+}: 4.20 mg dm^{-3}; Zn^{2+}: 0.70 mg dm^{-3}; Sand: 92.9 dag kg^{-1}; Silt: 3.5 dag kg^{-1}; Clay 3.6: dag kg^{-1}; Bulk density: 1.45 g cm^{-3}; Particle density: 2.66 g cm^{-3} and Total porosity: 0.45.

Organic wastes were mixed with soil after drying in the sun, in proportions (v/v, waste:soil) such as 0:100; 20:80; 40:60; 60:40; 80:20, and 100:0. The experiment was conducted as factorial arranged in a completely randomized design with 10 replications. We also used an additional treatment consisting of the following composition: 700 L of subsurface soil corrected with 300 L of cattle manure, 5 kg of superphosphate, and 0.5 kg of potassium chloride (Carvalho et al., 1978).

Jatobá seeds were collected from different indigenous matrices located in the city of Bom Jesus. Before sowing, seeds were kept in sulfuric acid (95–97%) for 1 h (chemical scarification) to break dormancy, and they were washed for 10 min under running tap water (Dechoum, 2004). Three seeds each were seeded in perforated plastic bags with a capacity of 1 kg.

The emergence percentage (E%) was calculated according to Labouriau and Valadares (1976), the formula below is used, %E = (N/A) x 100; where: N is the total number of emerged seeds and A is the total number of seeds germinated. The emergence rate index (ERI) was calculated using the formula (Maguire, 1962), ERI = åNi/Di, wherein Ni is the number of germinated seeds, and Di is days after planting

After 108 days of seeding, the following parameters in the seedlings were evaluated: the height (SH) of a ruler, considering as standard yolk terminal (apical meristem), stem diameter (SD) measured with a precision caliper (± 0.05 cm), and the relationship between seedling height and stem diameter (SH/SD). Subsequently, the aerial part dry weight (ADW) and root dry weight (RDW), being evaluated 5 randomly selected seedlings from each treatment. Samples were dried at 60°C for 72 h by using a forced air circulation drier. ADW and RDW were added to obtain the total dry weight (TDW). The shoot samples were ground in a Willey TE-650 mill to determine the macronutrient content in the plants (Malavolta, 1997): (i) N, determined by the Kjeldahl method in which

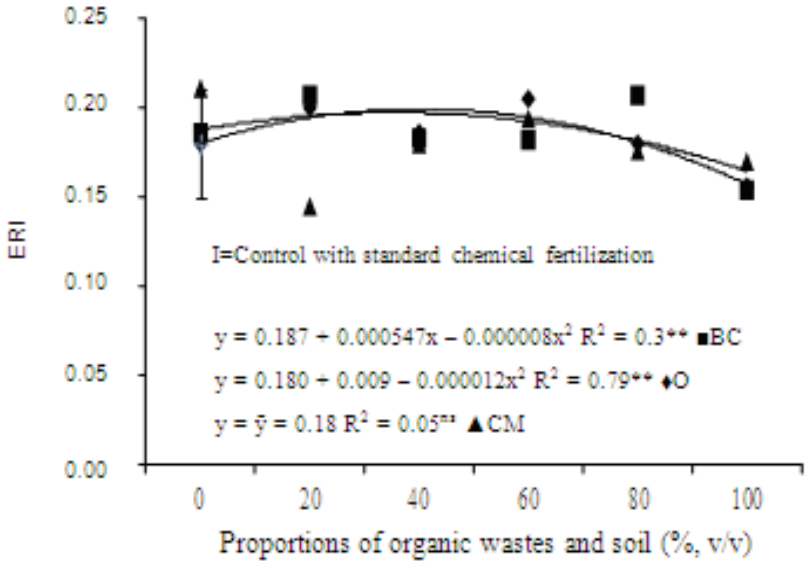

Figure 1. Emergence rate index (ERI) of seedlings of *Hymenaea stigonocarpa* Mart with different compositions of substrate (■ BC: bagana of carnaúba; ♦ O: compost; ▲ CM: cattle manure). I: standard error.

the extracts were prepared from solutions by digestion with sulfuric acid; (ii) P, determined by colorimetry; (iii) K, determined by flame emission photometry; (iv) Ca, determined by EDTA titration. The accumulation of each element in the shoots of seedlings was calculated as the product of dry weight and the content of nutrients. We calculated the relationships between seedling height and aerial part dry weight (SH / ADW) and aerial part dry weight and root dry weight (ADW / RDW). Dickson's quality index (DQI) was calculated using the following formula: DQI = TDW (g) / [SH (cm) / SD (mm) + ADW (g) / RDW (g)] (Dickson et al., 1960).

The results were subjected to analysis of variance Scott-Knott test mean 5% for the sources of organic waste that characterized the qualitative treatments and regression polynomial with respect to the proportions of bagana of carnaúba, cattle manure, and compost (quantitative treatments). The interaction between the sources of organic waste in the different proportions of bagana of carnaúba, cattle manure, and organic compost were determined. The statistical program SISVAR 4.2 was used to analyze the data (Ferreira, 2011).

RESULTS AND DISCUSSION

The addition of organic residues in different proportions of soil did not affect the E% of jatobá seedlings. On the basis of ERI, it was observed that there was an interaction between the sources of organic waste and the proportions of soil wherein the estimated highest was obtained in the organic compound at a ratio of 40:60 (organic compost: soil), with a quadratic response (Figure 1). The substrates composed of organic compounds probably have adequate hydric availability, thus providing the conditions necessary to maximize seed germination and subsequent seedling emergence. The effect of adding organic compost to jatobá seedlings was also observed by Santos et al. (2011) in their study, in which seedlings were grown in a greenhouse by using a commercially made 100% organic compost. They obtained an ERI of 0.89, which was equivalent to the substrates with a higher index. Araújo and Sobrinho (2011) evaluated the germination and seedling production of *Enterolobium contortisiliquum* on different substrates; they found better performance in the ERI of the substrate containing soil, cattle manure, and carbonized rice hull. According to the authors, the ERI may have been influenced by the materials that retain water in adequate quantity, thereby suggesting that the reference substrates promoting seedling emergence, probably due to the less physical impediment to emergence, may have also occurred in this study.

There was an interaction between the sources of organic waste and soil ratios for the following variables: SH, SD, SH/SD, ADW, RDW, TDW, SH/ADW, ADW/RDW, and DQI. The highest SH (31.04 cm plant^{-1}) was obtained for seedlings grown on substrates with bagana of carnaúba in the estimated proportion of 56:44 (bagana:soil), which was higher than the seedling grown with a standard fertilizer (24.2 cm·plant^{-1}). Seedlings grown in soil with compost showed an increasing linear effect, while those grown in soil with manure exhibited a decreasing linear effect (Figure 2a). Costa et al. (2005) also investigated the effect of bagana of carnaúba (50% bagana of carnaúba + 50% commercial vermiculite) on the SH of *A. muricata* seedlings and obtained a mean of 27.7 cm·plant^{-1}. This positive influence of bagana can be attributed to the greater availability of nutrients provided by it, which can be explained on the basis of the

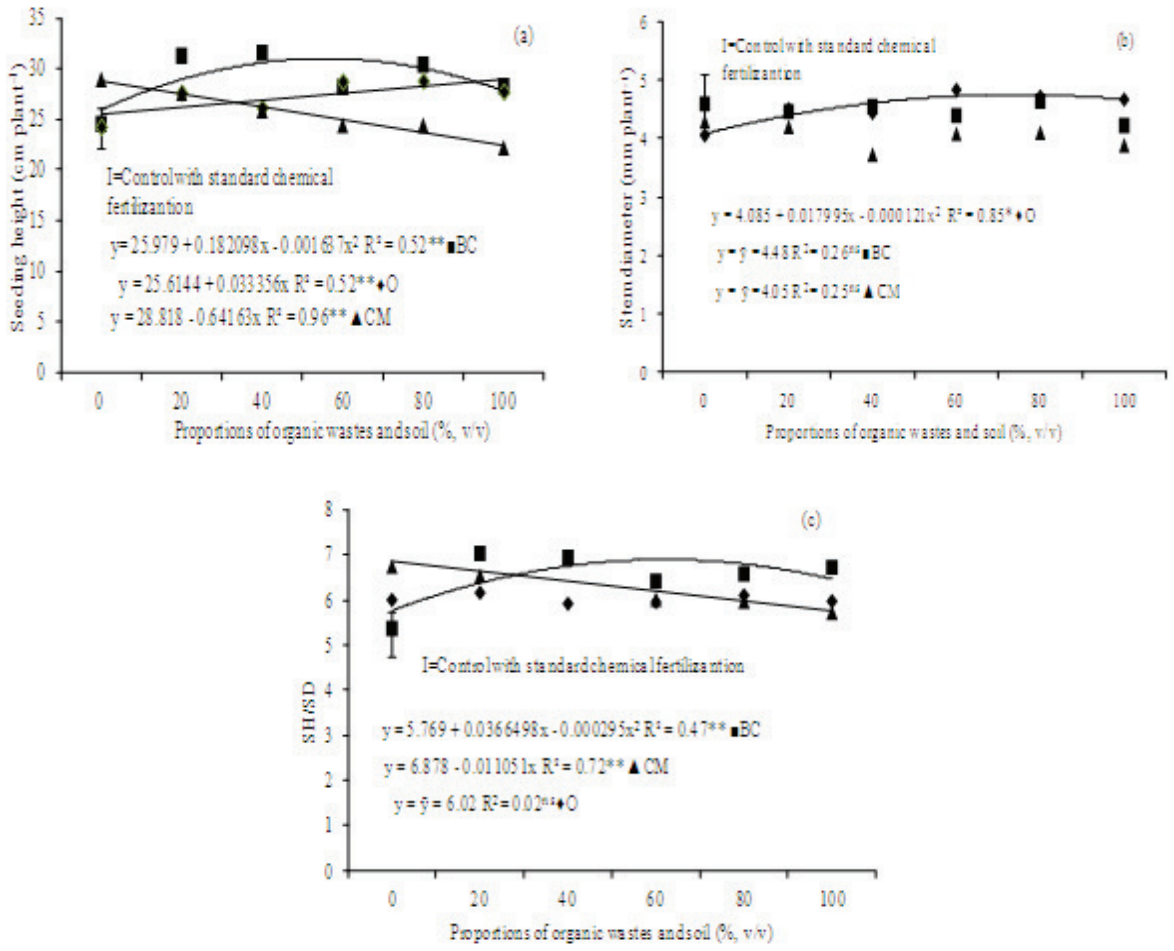

Figure 2. Seeding height (SH) - (a), stem diameter (SD) - (b) and relationship between seeding height and stem diameter (SH/SD) - (c) of seedlings of *Hymenaea stigonocarpa* Mart with different compositions of substrate (■ BC: bagana of carnaúba; ♦ O: compost; ▲ CM: cattle manure). I: standard error.

observation that seedlings grown on a substrate with a dose of 0:100 (bagana:soil) showed the lowest SH (24.56 cm·plant^{-1}).

However, the seedlings grown on a substrate containing organic compounds in the ratio 75:25 (compost:soil) showed the highest (4.75 mm·plant^{-1}) SD value. The seedlings grown with a standard fertilizer showed an SD (4.61 mm plant^{-1}) within the confidence interval, while those grown with substrates containing an increased proportion of bagana of carnaúba and cattle manure had no significant effects (Figure 2b). Santos et al. (2011) cultivated seedlings of the same species in a greenhouse using 70% commercial organic manure; they obtained an SD value higher than that observed in this study (5.90 mm), but measurements were taken 120 days after sowing. Carvalho Filho et al. (2003) found a better SD in the seedlings cultivated in substrate mixtures containing sand and manure, suggesting the requirement of the species by mixing lighter.

The highest mean SH/SD ratio (6.89) was obtained for the seedlings grown with the estimated proportion of 62:38 (bagana:soil), which was also higher than the seedlings grown with a standard fertilizer (5.26 plant^{-1}). This ratio was within the limit (the highest ratio of 10) obtained by Birchler et al. (1998). Seedlings grown in substrates containing organic compounds had no significant effects, while those grown in substrates containing manure showed a decreasing linear effect (Figure 2c). According to Silva et al. (2007), this is an important feature for the successful adaptation of plants in the fields, because the lower the ratio, the more the plants are resistant to environmental conditions because of the greater balance between the aboveground biomass and roots.

Biomass, ADW, RDW, and TDW showed a quadratic effect with respect to the proportions of bagana and compost, while the seedlings grown with manure showed a negative linear effect (Figure 3a-c). The ADW production was 4.11 and 3.94 g·plant^{-1} for the estimated proportions of 49:51 (bagana:soil) and 53:47 (organic

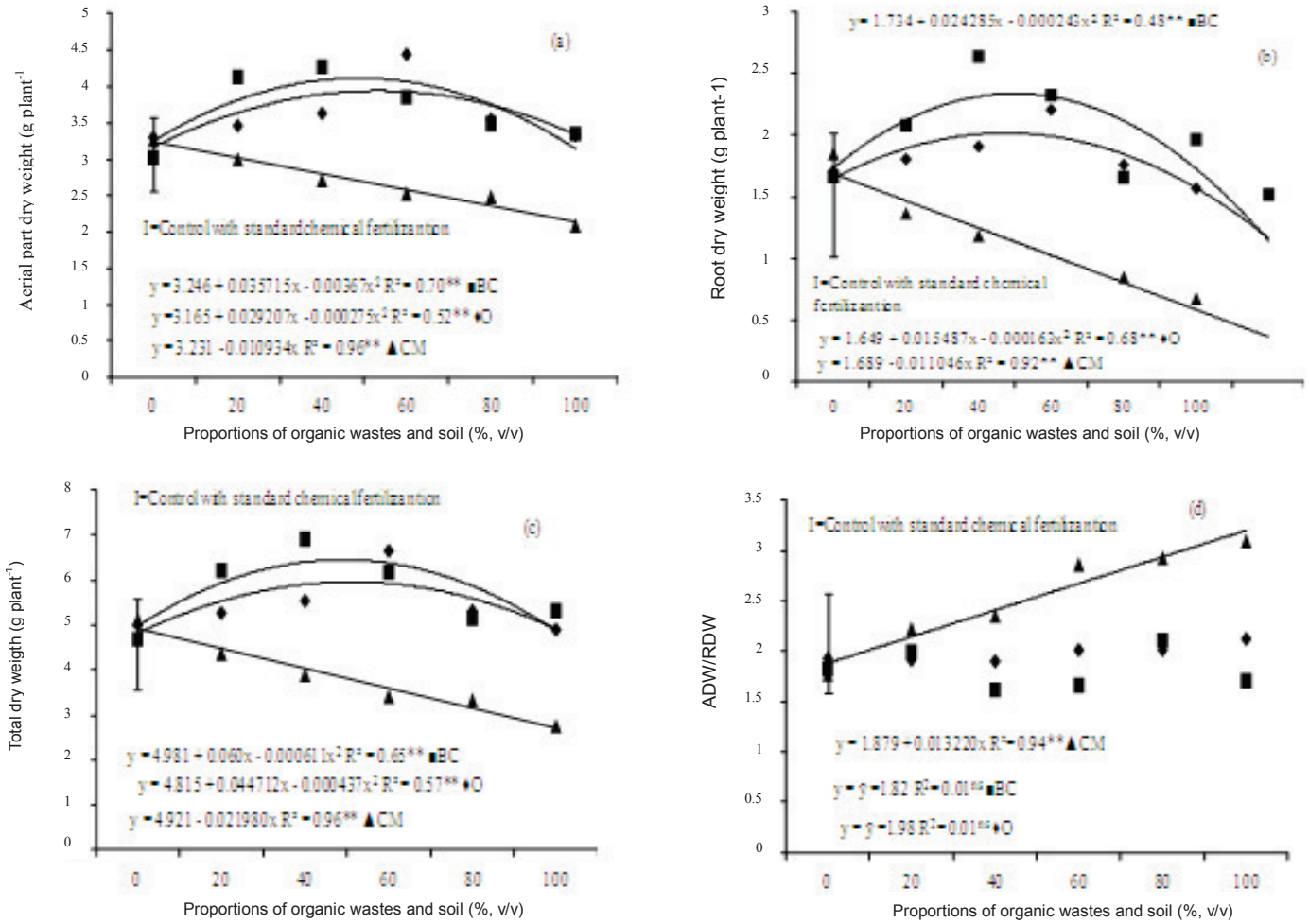

Figure 3. Aerial part dry weight (ADW) - (a), root dry weight (RDW) - (b), total dry weigth (TDW) - (c) and relationship between the aerial part dry weight and the root dry weight (ADW/RDW) - (d) of seedlings of *Hymenaea stigonocarpa* Mart with different compositions of substrate (■ BC: bagana of carnaúba; ♦ O: compost; ▲ CM: cattle manure). I: standard error.

compost:soil), respectively. These values were higher than those for the seedlings grown with a standard fertilizer (3.26 g·plant^{-1}) (Figure 3a). Costa et al. (2005) also indicated an increase in this variable in 25% of *A. muricata* seedlings with the addition of bagana, obtaining a highest yield (3.79 g·plant^{-1}) after 140 days of sowing. Arthur et al. (2007) evaluated the effect of cattle manure on the seedling formation of *Calophyllum brasiliense* Cambess; they found a reduction in this variable with an increase in the proportion of manure. According to them, manure is an important component of the substrate because it increases the organic matter content and cation exchange capacity of the substrate, and therefore, its use cannot be discontinued (although the dose can be adjusted).

The highest yields of RDW (2.34 and 2.04 g·plant^{-1}) were obtained in the substrates with the proportions of 50:50 (bagana:soil) and 48:52 (organic compost:soil), respectively, while the RDW yield of superior seedlings grown with a standard fertilizer was 1.52 g·plant^{-1} (Figure 3b). Costa et al. (2005) described the effect of bagana of carnaúba on this variable in their study on the seedlings of *A. muricata* using 33% bagana. The increase in RDW on basis of the doses of bagana and organic compost can be attributed not only to the improved chemical properties but also to the physical properties that probably allowed better root development.

The highest values of TDW (6.45 and 5.95 g·plant^{-1}) were obtained in the substrates with the estimated proportions of 49:51 (bagana:soil) and 51:49 (organic compost:soil), respectively, while the seedlings grown with a standard fertilizer yielded 4.59 g·plant^{-1} (Figure 3c). The substrates containing bagana of carnaúba significantly influence TDW, as well as SH, and SH/SD (Figure 2a and c), ADW, and RDW (Figure 2a and b, respectively). The estimated dose of 49:51 (bagana:soil) promoted an increase in TDW (29.31%) compared to the dose of 0:100 (bagana:soil). The decreasing trend in the

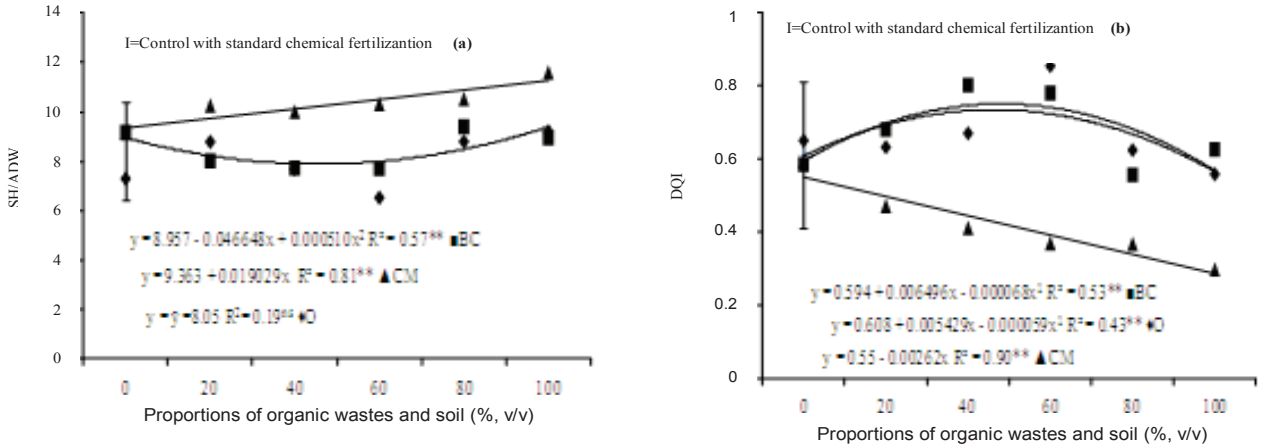

Figure 4. Relationship between seedlings height and aerial part dry weight (SH/ADW) - (a) and e Dickson quality index (DQI) - (b) of seedlings of *Hymenaea stigonocarpa* Mart with different compositions of substrate (■ BC: bagana of carnaúba; ♦ O: compost; ▲ CM: cattle manure). I: standard error.

function of different doses of manure on TDW was also confirmed by Arthur et al. (2007), who obtained the highest yield (17.13 g·plant^{-1}) in the absence of manure in the seedlings of *C. brasiliense*. Since mineralization of the manure added to the soil may probably not be adequate, the organic acids derived from decomposition might have caused a detrimental effect on the seedling biomass production.

The ADW/RDW relationship was influenced by the addition of cattle manure, which increased linearly. Seedlings cultivated in the ratio of 100:0 (manure: soil) showed a higher mean value (3.09 plant^{-1}) than the seedlings grown with a standard fertilizer (2.08 plant^{-1}). The seedlings cultivated with organic compost and bagana showed no significant effects (Figure 3d). Caldeira et al. (2008) found the ADW/RDW relationship in seedlings to be 2:1. According to these authors, it is important to analyze this relationship when the seedlings are in the field, because SH should be lower than the root length, as a function of possible problems regarding water uptake to the shoot. In this study, only the previously cited dose was higher than this ratio, with the others being below 3:1.

SH/ADW showed a quadratic effect in relation to the proportion of bagana and soil, with the lower mean (7.89) for the seedlings cultivated in the estimated proportion of 46:54 (bagana:soil). The substrates containing compost showed no significant effects in the SH/ADW relationship, while those containing manure showed a linear increase in SH/ADW values with the highest mean (9.24), compared to the seedlings grown with a standard fertilizer (8.42) (Figure 4a). Gomes et al. (2002) assessed the morphological parameters of the seedlings of *Eucalyptus grandis* and found that the SH/ADW ratio showed the greatest relative contribution, indicating its importance despite taking into account a destructive parameter, the weight of dry matter. In this study, all mean values were higher than the mean recommended by Brissete and Barnett (1991), which is too independent of the species.

DQI showed a quadratic effect in relation to the dose of bagana of carnaúba and organic compost, with the highest values (0.75 and 0.73) obtained in the seedlings cultivated in the estimated proportions of 46:54 (bagana:soil) and 48:52 (organic material: soil), respectively, compared to the seedlings cultivated with a standard fertilizer (0.61). With the increase in the proportion of manure in the substrate, a linear decreasing effect was observed in DQI (Figure 4b).

Seedlings cultivated with bagana showed higher values of DQI and also exhibited higher levels of SH, SH/SD, ADW, RDW, and TDW. According to Bernardino et al. (2005), the higher these values, the better the quality of the seedlings. The decrease in SH, SH/SD, ADW, RDW, TDW, and DQI in the seedlings of *H. stigonocarpa* with the addition of cattle manure to the substrate contradicts the observations found in other forest species. This may have occurred because the manure may not have fully decomposed, and consequently the nutrients may not have been readily available to the seedlings, because of the presence of organic acids present in plant materials that comprise the organic compost.

Several authors recommend the DQI as an index to determine the quality of seedlings (Fonseca et al., 2002; Nóbrega et al., 2008; Costa et al., 2011). In this study, we observed that a higher DQI (0.75) was obtained for the estimated dose of 48:52 (bagana:soil), which is the most recommended dose for the production of seedlings of *H. stigonocarpa*.

The addition of organic compost, mainly bagana, promoted an increase in the growth variables in relation to the dose of 0:100 (organic waste: soil). This can be observed with the increments of 27.12 and 12.3% in the estimated proportions of DQI 48:52 (bagana:soil) and

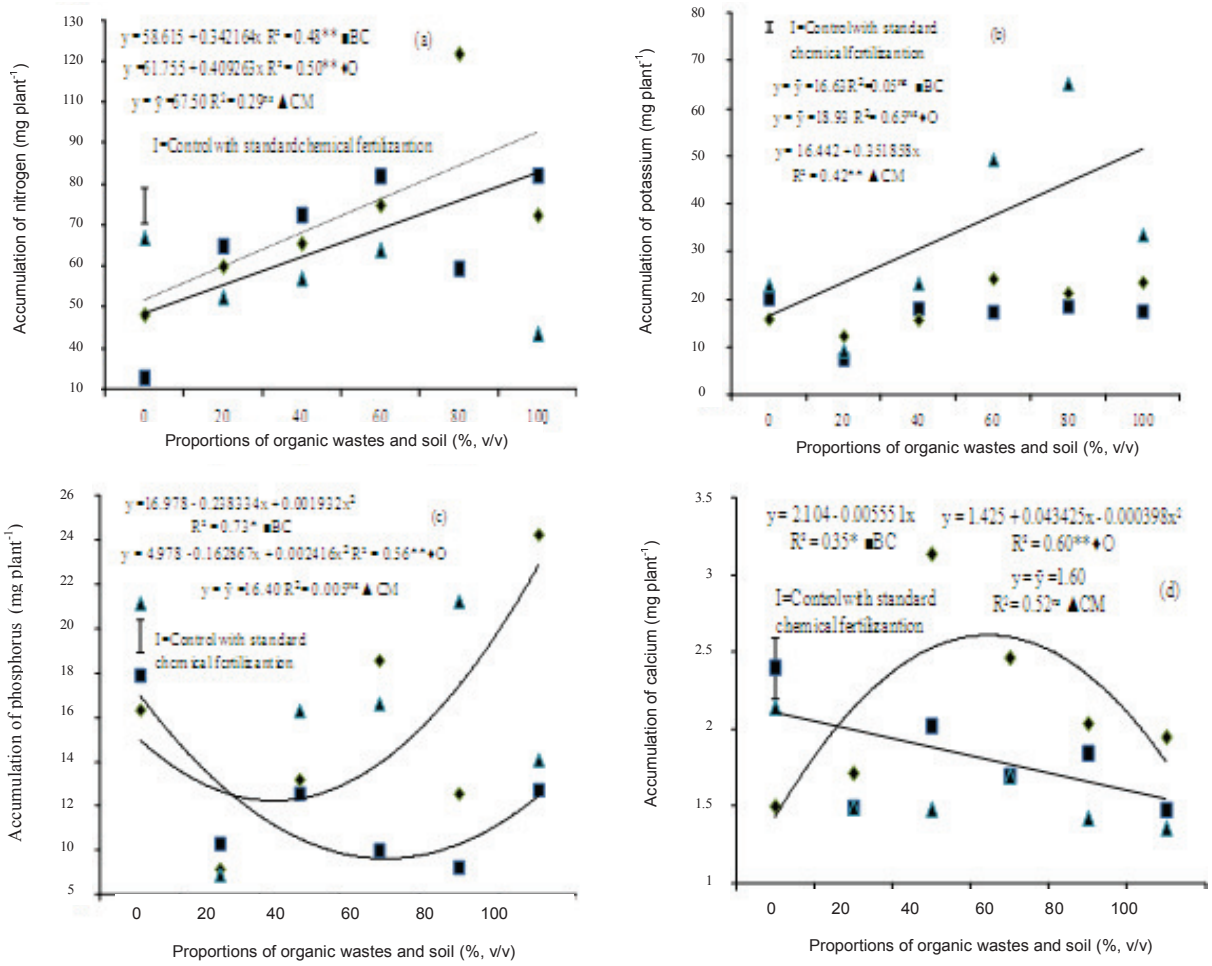

Figure 5. Accumulation of nitrogen (N) - (a), potassium (K) (b), phosphorus (P) - (c) e calcium (Ca) - (d) in aerial part dry weight of seedlings of *Hymenaea stigonocarpa* Mart with different compositions of substrate (■ BC: bagana of carnaúba; ♦ O: compost; ▲ CM: cattle manure). I: standard error.

46:54 (organic compost: soil), respectively, indicating that *H. stigonocarpa* is responsive to the nutrients added to the soil. The response of *H. stigonocarpa* to the addition of organic residues to the soil was also observed by Costa et al. (2011); they observed that the seedlings grown in a greenhouse substrate with coconut fiber had a greater DQI.

There was an interaction between the sources of organic waste and soil ratios caused by the accumulation of N, K, P, and Ca in the ADW of seedlings, excluding Mg. The accumulation of N in the ADW of seedlings was influenced by the quantity of organic compost and bagana of carnaúba added to the substrate, which showed an increasing linear effect. Seedlings cultivated in the ratio of 100:0 (organic compost: soil) showed a higher mean (102.68 mg·plant^{-1}), compared to the seedlings cultivated with a standard fertilizer (84.84 mg·plant^{-1}). The seedlings cultivated with cattle manure showed no significant effects (Figure 5a). The addition of organic compost increased the accumulation of N in the ADW with respect to the dose of 0:100 (organic compost: soil). This can be observed in the increase (66.27%) in N content at a dose of 100:0 (organic compost:soil). Souza et al. (2005) evaluated the effect of different substrates on the production of ipê amarelo [*Tabebuia serratifolia* (Vahl.) Nich.] seedlings for 270 days and found that, compared to the level of N in the shoots of seedlings cultivated in the substrate subsoil, the level of N was higher in those cultivated in the substrate containing organic compost (plant remains, 70%; cattle manure, 25%; and chicken manure, 5%).

The accumulation of K in the ADW of seedlings increased linearly when cattle manure doses were added to the substrate, with the highest mean (51.63 mg plant^{-1}) obtained in the ratio of 100:0 (manure:soil). The seedlings grown using a standard fertilizer showed greater accumulation of K (77.31 mg·plant^{-1}), while those grown in substrates with a higher dose of bagana and organic compost showed no significant effects (Figure 5b). Severino et al. (2008) evaluated the macronutrient

content in castor seedlings cultivated in five organic substrates and found that seedlings grown in substrates containing cattle manure showed the highest levels of K. According to Duboc et al. (1996), among the macronutrients, K is the least required element by jatobá.

The accumulation of P in the ADW of seedlings showed a quadratic effect in relation to the levels of organic compost and bagana, with the highest accumulation (12.23 and 9.63 mg·plant^{-1}) in the estimated proportions of 34:66 (bagana:soil) and 62:38 (organic waste:soil); however, seedlings grown with fertilizers were inferior (19.7 mg·plant^{-1}). The substrates with increased manure content showed no significant effects (Figure 5c). Trindade et al. (2001) evaluated the nutrition of *E. grandis* seedlings in response to organic compost; they reported that P uptake was always a function of increasing doses of compost-based manure and straw grass, with the largest increase occurring between doses of 0 and 5%. According to these authors, P is a very important nutrient for plant growth even in low doses; however, the availability of this nutrient is limited in the soil under natural conditions and needs to be supplied externally.

The accumulation of Ca in the seedlings cultivated with substrates containing organic compost showed a higher mean (2.61 mg·plant^{-1}) in the estimated proportion of 54:46 (organic compost:soil), which was within the confidence interval of seedlings grown with a standard fertilizer (2.4 mg·plant^{-1}). Seedlings grown in substrates containing bagana of carnaúba showed a decreasing linear effect, while those containing cattle manure showed no significant effects (Figure 5d). Trindade et al. (2001) conducted nutrition studies on *E. grandis* seedlings in response to organic compost, and they reported that the absorption of Ca was always a function of increasing doses of compost-based manure and straw grass, with the largest increase occurring between doses of 0 and 5%. Duboc et al. (1996) evaluated the nutrition of jatobá and observed that the Ca content in ADW of the treatment without Ca did not differ from the Ca content in the treatment with Ca; this suggests that jatobá has a high capacity to extract calcium from the substrate, even under limited availability or low physiological requirement for this nutrient.

Conclusions

The addition of bagana of carnaúba, organic compost, and cattle manure in soil samples did not influence the emergence of *H. stigonocarpa* seedlings. The estimated proportion of 40:60 (organic compost:soil) allows a greater speed of emergence of seedlings. The addition of bagana of carnaúba promotes increase in height, ratio of height by diameter, aerial part dry weight, root dry weight, total dry weight, ratio of aerial part height by aerial part dry weight, and the Dickson quality index of *H. stigonocarpa* seedlings, in the estimated proportion of 48:52 (bagana of carnaúba:soil), which is the most suitable substrate composition for the cultivation of this species. Seedlings cultivated with 100% cattle manure had higher K accumulation in the shoots. The substrate containing organic compost enabled a greater increase in the accumulation of N, P, and Ca in the shoots of jatobá seedlings, possibly because of the greater availability of these nutrients.

Conflict of Interest

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

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Full Length Research Paper

Effects of pre-sowing treatments on *Jatropha curcas* seed germination and seedling growth

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This study aimed to investigate the effects of different pre-sowing treatments on *Jatropha curcas* seed germination rate and seedling early growth. Growth and vigour of the seedlings was assessed through the measurements of growth parameters, in order to identify the best pre-sowing treatment, which guarantees both the highest seed germination rate and the best development and growth of the seedlings. *J. curcas* seeds of the 'Indian' cultivar were collected in Tamale region (Ghana) and subjected to five different pre-sowing treatments: i) control; ii) soaking in 30°C water for 24 h; iii) hammer shell cracking; iv) warm stratification at 37°C for 24 h; v) hammer shell cracking plus warm stratification at 37°C for 24 h. Amongst the sixteen indices considered in the experiment (six germination indices and ten growth rate indices), results revealed that the tested pre-sowing treatments influenced much more seed germination than seedling growth. Shell cracking treatment enhanced seed germination and warm stratification promoted emergence rate and seedling growth as compared to the other tested treatments.

Key words: Physic nut, biodiesel, rural development, vegetable oil, land use.

INTRODUCTION

Jatropha curcas L., a drought avoidant perennial small tree, is autochthonous of Mexico and tropical America, and was then largely spread out in India, Africa and South East Asia (Achten et al., 2010a). Nowadays, *J. curcas* grows in tropical and subtropical regions in a wide range of climatic conditions from semiarid to humid (Achten et al., 2010a). In the last decades, *J. curcas* has become popular thanks to its wide capabilities and plethora of uses, including biodiesel production, which are the cause of an increasing of hectares of *J. curcas* yearly planted at global level (Fairless, 2007; Kant and Wu, 2011). *J. curcas* seeds contain about 25 to 35% or more of oil (Freitas et al., 2011; Verma and Verma, 2014), which can be extracted and used as lighting and cooking fuel, to manufacture soap, medicine or bio-pesticide and, after further chemical treatments, to produce biodiesel, a renewable energy source alternative to conventional petrodiesel (Martínez-Herrera et al., 2006; Pompelli et al., 2010; Contran et al., 2013; Sunil et al., 2013; Sushma, 2014). Besides the economic value derived from *J. curcas* oil and its derived products, *J. curcas* strength as a crop derives from its potential

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adaptability to grow on low-nutrient soils and under arid and semi-arid conditions, avoiding *J. curcas* competition against food crops. Furthermore, the plant itself offers the ecological advantage to mitigate soil degradation and to restore marginal land or abandoned farmland (Reubens et al., 2011).

Nevertheless the positive impacts that could be generated by the use of *J. curcas* in arid and semi-arid areas of developing countries, the high potential of this tree has not been reached so far and *J. curcas* is still a (semi-) wild undomesticated plant. Its basic agronomic needs are only partially understood, the growing and management practices are not enough documented for a lot of areas of new introduction, and the environmental effects should more deeply investigated (Achten et al., 2010a, b, c; Contran et al., 2013; Yamada and Sentelhas, 2014). Studies on vegetative (cutting) or generative (seed) propagation of *J. curcas*, representing a critical stage in the plant-life cycle, have been carried out so far (Ginwal et al., 2005; Achten et al., 2008; Kumar and Sharma, 2008; Islam et al., 2009; Severino et al., 2010; Windauer et al., 2012; Moncaleano-Escandon et al., 2013).

High variability of seed germination has been recorded as influenced by the observed genotype (cultivar, seedling or population), time after harvest and storage conditions, environmental characteristics of plant growing, pre-sowing and after-sowing treatments (temperature and water potential of seed tissues and substrates) (Islam et al., 2009; Pompelli et al., 2010; Windauer et al., 2012; Duong et al., 2013; Moncaleano-Escandon et al., 2013). Some authors report on a loss of seed viability and germinability after medium and long term storage (Duong et al., 2013; Moncaleano-Escandon et al., 2013), while others suppose that the presence of seed coat, may be the responsible of a physical dormancy, and furthermore generates the need to remove this inhibition by pre-sowing treatments (Baskin and Baskin, 1998; Islam et al., 2009; Windauer et al., 2011).

In order to enhance germination percentage, seeds have been subjected to pre-germination treatments before sowing, with the aim to break the seed coat, favour the embryo hydration and consequently increase the germination percentage as compared to untreated seeds. Among the studies on the effects of pre-sowing treatments of *J. curcas* seeds on different germination parameters, Islam et al. (2009) demonstrated that *J. curcas* seeds, kept under stone sand and moistened with water for 72 h before sowing, showed a significantly higher germination percentage than the untreated and directly sown control in all the twenty different genotypes tested in the experiment. Windauer et al. (2012) tested the effects of different temperatures (from 15 to 35°C) on *J. curcas* seed germination percentage. This study revealed that an incubation of seeds at 25°C before sowing caused the highest final germination percentage, even if at 30°C seeds germinated faster than any other temperature. Furthermore, positive results were reached for seed of various tropical tree species, previously treated with hot water, which is considered one of the cheapest, easiest and replicable techniques to induce seed dormancy-breaking (Wang and Hanson, 2008). Only few studies were found in literature on the effects of pre-sowing treatments on the growth of *J. curcas* seedlings (Islam et al., 2009; Pompelli et al., 2010; Moncaleano-Escandon et al., 2013).

The aim of this study was to investigate the effects of different pre-sowing treatments on germination behaviour of the seeds of 'Indian' cultivar, which in spite of the agronomic value showed some difficulties to obtain a good rate of propagation. Growth rate and vigour of the seedlings through the measurements of growth parameters were also assessed.

MATERIALS AND METHODS

The experiment was performed in a growth chamber of the Department of Agriculture of the University of Sassari (Italy) and carried out on *J. curcas* seeds of the 'Indian' cultivar. This cultivar has been chosen, since it is one of the most common cultivar used in Ghana and India and largely adopted in many countries for small-scale extensive plantations, promoted by cooperation projects, and large-scale intensive plantations, operated by multinational companies (Acheampong and Campion, 2014). *J. curcas* seeds were collected in October 2011 from Ghana Yendi road Farm, Tamale, Northern Region of Ghana. The area of Tamale is classified as a tropical savannah climate zone (Peel et al., 2007), characterized by a pronounced dry season (from October to March), in which precipitation is less than 60 mm. The average annual precipitation is 1179 mm (MOFA, 2011). The average annual temperature is 27.8°C (min 22.3°C - max 33.4°C) (Climatedata, 2014). Seed were stored at 18 (±2)°C and 75% relative humidity for six months until the start of the experiment (April, 2012).

J. curcas seeds were subjected to five different pre-sowing treatments as follows: i) untreated control, in which seeds were directly sown in pot in a depth of 1 cm; ii) seed soaking in 30°C water for 24 h; iii) hammer shell cracking, in which seeds were mechanically scarified by cracking with a hammer to weaken the shell; iv) warm stratification at 37°C for 24 h, in which seeds were mixed with an equal volume of a moist medium (peat) in a close container and maintained at 37°C for 24 h; v) hammer shell cracking plus warm stratification at 37°C for 24 h, in which seeds were mechanically scarified by cracking with a hammer to weaken the shell and then mixed with an equal volume of a moist medium (peat) in a close container and maintained at 37°C for 24 h. Germination test was carried out in a growth chamber at 28°C, under an 8/16 light/dark regime at 400 µmol m^{-2} s^{-1}. A completely randomised design with four replications per treatment was used, according to the Seeds Analysis Rules (ISTA, 2004). Seeds were sown in pot (15 cm diameter, 10 cm height) filled with potting mix medium (dry matter 30%, organic matter 20%, fertilizer NPK 12:14:24 1 kg/m^3) and fully irrigated every day with a total of 55 ml per pot of distilled water during the first two weeks of the experiment, 100 ml per pot during the period between 15 and 25 days and with 150 ml per pot between 26 and 35 days.

Number of emerged seeds and first true leaf expansion were recorded by everyday monitoring from the sown for 35 days. The seed emergence criterion was visible protrusion from the surface of

soil (AOSA, 1983). Emerged seed was considered germinated (Ranal and De Santana, 2006). According to Cornelissen et al. (2003), after 35 days from sown, seedlings developed by germinated seeds were separated into cotyledons, leaves, stem, and roots (washed). The following destructive measurements were carried out: i) cotyledons (fresh and dry weight and total cotyledons area), ii) leaf (fresh and dry weight and total leaf area), iii) stem (length, basal diameter, fresh and dry weight), and iv) root (length, diameter, fresh and dry). Dry weight was measured when samples at 100°C reached a constant weight (around 48 h). Total cotyledon area and leaf area were measured by an Area Meter (LI-3100C Area meter, Licor), which provided a scanner of the leaf blade and allowed further biometric determination by means of a specific software. Measured data on several parameters related to germination process and seedling growth were allowed for calculation, as reported in Table 1.

Data were checked for normal distribution (Shapiro-Wilk W test) and homogeneity of variance (Levene's test). Percent values were transformed by arcsine-square root prior to analysis. Analysis of variance (ANOVA) was applied to test the effect of Treatments. For the ANOVA of seed germination parameters, the statistical unit was the replication (N=4). For the ANOVA of seedling growth parameters, the statistical unit was the single seedling (N=14); since the number of germinated seeds in water soaking treatment was very low, this treatment was not considered for the ANOVA test of seedling growth parameters. A Tukey HSD test was applied to compare the above effects between homogeneous groups. Tests of significance were made at a $p \leq 0.05$ confidence level. Analyses were processed by using STATISTICA 6.0 Package for Windows (StatSoft, Inc. 2013).

RESULTS

The statistical analysis revealed that the tested pre-sowing treatments have various effects on different germination parameters of *J. curcas* seeds. In particular, germination ability, emergence rate, emergence index, and mean emergence time were different between treatments and control, while true leaf expansion and seedling vigour index did not revealed any difference (Table 2).

More in detail, water soaking treated seeds showed the lowest percentage of germinability (7.5%) while shell cracking treated seeds showed the highest percentage of germination (>80%) (Figure 1). Seeds exposed to shell cracking, warm stratification and the combination of these two pre-sowing treatments (shell cracking plus warm stratification) had higher emergence rate and, consequently, lower mean emergence time compared to control untreated seeds (Figure 1). Furthermore, the highest value in emergence rate (24 day^{-1}) was found in warm stratification treated seeds, which showed higher emergence rate also compared to shell cracking treated seeds (Figure 1). Both seeds treated with the sole shell cracking and with the combination of shell cracking plus warm stratification showed statistically higher emergency index than untreated control seeds, while warm stratification treated seeds did not showed a significant difference from control, shell cracking, and shell cracking plus warm stratification treated seeds (Figure 1). True leaf expansion and seedling vigour index do not provide significant differences among treatments. However, these data have been reported for a complete information on the experiment results, as well to corroborate the good performance of the warm stratification in the case of the true leaf expansion and of the shell cracking and warm stratification in the case of the seedling vigour index.

After 35 days from the sown, amongst the ten growth parameters, only cotyledon size, leaf size, and specific root length showed significant differences among treatments (Table 3). Since the number of germinated seeds in water soaking treatmet was very low (7.5% on 40 seeds), this treatment was not considered for the analyses of seedling growth parameters.

Warm stratification treated seedlings showed the highest cotyledon size, which is significantly higher than in shell cracking plus warm stratification treated seedlings (Figure 2). Maximum leaf size was observed in warm stratification treated seedlings, which also revealed significant differences, as compared with control, shell cracking and shell cracking plus warm stratification treated seedlings (Figure 2). Any significant difference among treatments was observed for specific cotyledon area and cotyledon dry matter content. These data, however, were reported as complementary information, as well as specific leaf area and leaf dry matter content that are in accordance with the highest leaf size of the seedlings of the warm stratification treatments.

Shell cracking plus warm stratification treated seedlings showed the highest specific root length values amongst the five tested pre-sowing treatments (Figure 3). Stem specific density, stem dry matter content and root:shoot ratio do not provide significant differences among treatments and were showed for a complete information on the experiment findings.

DISCUSSION

Considering the strong variability and genotype dependence of previous experimental results obtained by many authors, our test focused on the effects of five different seed pre-sowing treatments on germination and growth of *J. curcas* seedlings of the commonly used 'Indian' cultivar. In fact, in spite of the large diffusion, few scientific data were recorded regarding the seed germinability of this cultivar.

Results of the present investigation indicated that among the pre-sowing treatments only shell cracking significantly influenced seed germination. With the exception of water soaking, treatments had a positive effect on the early growth and extension of *J. curcas* tissues, as demonstrated by a significant increasing of the emergence rate and the emergence index, and a significant reduction of the emergence time. We were expecting a much higher performance of soaking seeds in water at 30°C for 24 h, since Islam et al. (2009) demonstrated that *J. curcas* seeds soaked in water had a

Table 1. Estimation of parameters related to germination process and seedling growth.

Variable	Formula	References
Germination process		
Germinability (G) [%]	G = (Ng / N) * 100	(ISTA, 2004)
True leaf expansion (Tl) [%]	Tl = (Nl / G) *100	-
Emergence rate (Er) [day^{-1}]	Er = [ΣNd / (Σ D * Nd)] *100	Kotowski (1926)
Emergence index (EI) [day^{-1}]	EI = Σ (Nd / D)	AOSA (1983)
Mean emergence time (MET) [day]	MET = (Σ D * Nd) / ΣNd	Ellis and Roberts (1981)
Seedling vigor index (SVI) [cm %]	SVI = [(Ls+Lr) * G] / 100	Abdul-Baki and Anderson (1973)
Seedling functional traits		
Cotyledon size (Cs) [mm^2]	Cs = Ac	Cornelissen et al. (2003)
Specific cotyledon area (SCA) [mm^2 mg^{-1}]	SCA = Ac /DWc	Cornelissen et al. (2003)
Cotyledon dry matter content (CDMC) [mg g^{-1}]	CDMC = DWc / FWc	Cornelissen et al. (2003)
Leaf size (Ls) [mm^2]	Ls = Al	Cornelissen et al. (2003)
Specific leaf area (SLA) [mm^2 mg^{-1}]	SLA = Al /DWl	Cornelissen et al. (2003)
Leaf dry matter content (LDMC) [mg g^{-1}]	LDCM = DWl / FWl	Cornelissen et al. (2003)
Stem specific density (SSD) [mg cm^{-3}]	SSD = DWs / Vs	Cornelissen et al. (2003)
Stem dry matter content (SDMC) [mg g^{-1}]	SDCM = DWs / FWs	Cornelissen et al. (2003)
Specific root length (SRL) [cm mg^{-1}]	SRL = Lr /DWr	Cornelissen et al. (2003)
Root:shoot ratio (RSr)	RSr = DWr / DWc+l+s	-

Legend: D = number of days counted from the beginning of germination; N = total number of seed; Ng = total number of germinated seeds; Nd = number of seeds germinated on day D after sowing; Nl = number of expanded true leaf when seed is germinated; N_5 = number of seeds germinated on day 5 after sowing; N_{15} = number of seeds germinated on day 15 after sowing; Ls = average stem length (cm); Lr = average root length (cm); Ac = cotyledon area [mm^2]; DWc = cotyledon dry weight [mg]; FWc = cotyledon fresh weight [g]; Al = leaf area [mm^2]; DWl = leaf dry weight [mg]; FWl = leaf fresh weight [g]; DWs = stem dry weight [mg]; FWs = stem fresh weight [g]; DWr = root dry weight [mg]; DWc+l+s = cotyledons plus stem plus leaf dry weight [g].

Table 2. *F* values of one-way analysis of variance for the effects of treatment on germination process.

Dependent variable	Treatment
d.f.	4
Germinability	6.7**
True leaf expansion	1.2^{ns}
d.f.	3
Emergence rate	22.6***
Emergence index	7.7*
Mean emergence time	10.9***
Seedling vigor index	1.9^{ns}

*p ≤ 0.05, **p ≤ 0.01,*** p ≤ 0.001, ns = p > 0.05 (not significant). d.f. represents the degrees of freedom.

significantly higher germination than control due to the rupture of seed coat. Anyway, water soaking treatment caused a significant seed germinability reduction as compared to all the other treatments. It is possible to suppose that for the 'Indian' cultivar this practice may be effective only in an earlier or later time of application with respect to the six months of seed storage applied before treatments.

Seedling dry matter, nutrient allocation, and plant structural strength seem to be not affected by treatments (Figure 2). On the contrary, warm stratification at 37°C induced an increase in aboveground seedling growth since, after 35 days from the sown, seedlings showed a significantly higher expansion of both cotyledon and primary leaf size. Furthermore, in shell cracking plus water stratification treated seedlings, an antagonistic

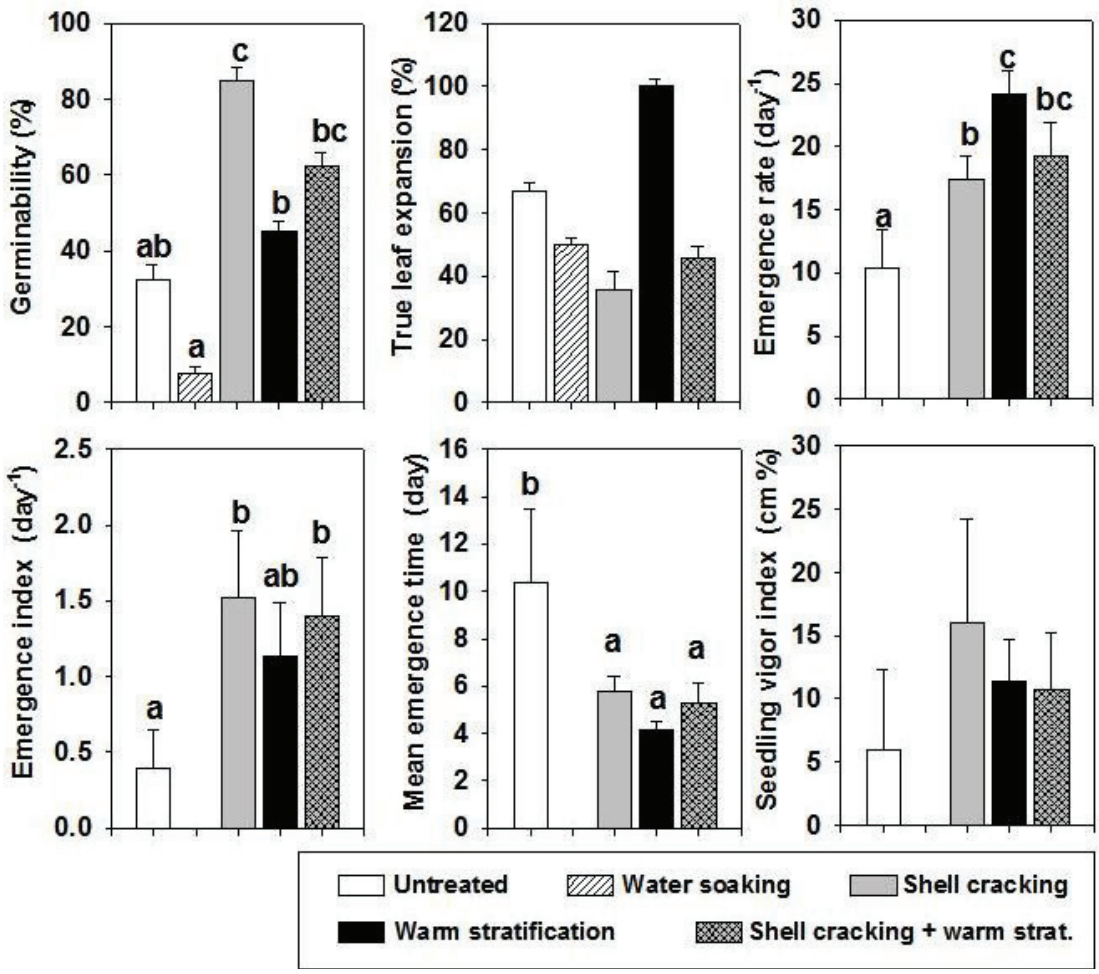

Figure 1. Germinability, true leaf expansion, emergence rate, emergence index, mean emergence time, and seedling vigour index of *J. curcas* seedlings growing after different pre-sowing treatments: untreated control, soaking in 30°C water for 24 h, shell cracking, warm stratification at 37°C for 24 h, and shell cracking plus warm stratification at 37°C for 24 h. Values represent means ±S.D. (N=4). When Treatment factor is significant, different letters show significant differences among means (Tukey HDS test, $p \leq 0.05$, N=4).

Table 3. *F* values of one-way analysis of variance for the effects of treatment on *J. curcas* seedling growth.

Dependent variable	Treatment
d.f.	4
Cotyledon size	6.1**
Specific cotyledon area	0.5^{ns}
Cot. dry matter content	1.7^{ns}
Leaf size	6.6***
Specific leaf area	1.8^{ns}
Leaf dry matter content	0.9^{ns}
Stem specific density	0.4^{ns}
Stem dry matter content	1.6^{ns}
Specific root length	3.6*
Root:shoot ratio	2.1^{ns}

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ns = $p > 0.05$ (not significant). d.f. represents the degrees of freedom.

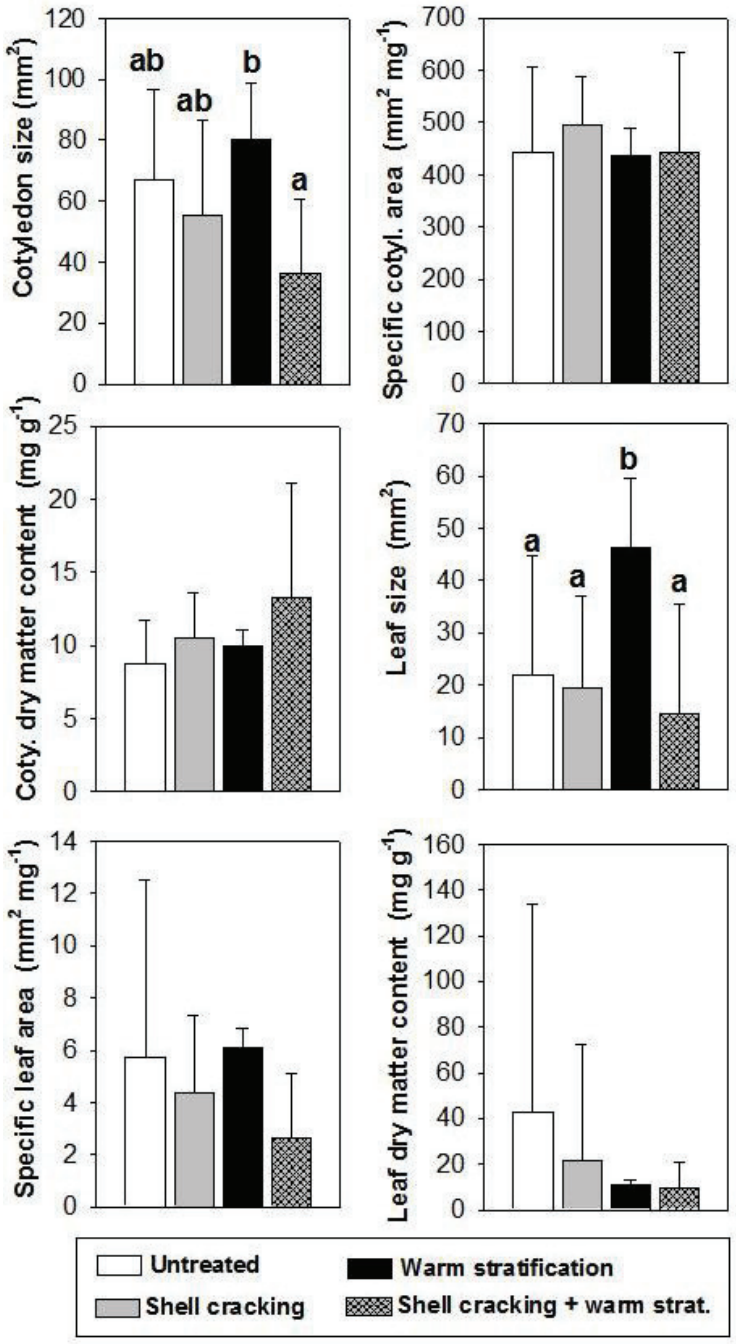

Figure 2. Cotyledon size, specific cotyledon area, cotyledon dry matter content, leaf size, specific leaf area, and leaf dry matter content of *J. curcas* seedlings growing after different pre-sowing treatments: untreated control, soaking in 30°C water for 24 h, shell cracking, warm stratification at 37°C for 24 h, and shell cracking plus warm stratification at 37°C for 24 h. Values represent means ±S.D. (N=14). When Treatment factor is significant, different letters show significant differences among means (Tukey HDS test, $p \leq 0.05$, N=14).

effect of the combined treatment on the aboveground part growth of the seedlings was observed, while an additive effect on root system growth was found (Figures 2 and 3). No correlation was found between germination and seedling growth indicators, thus showing some differences with respect to previous observations (Islam

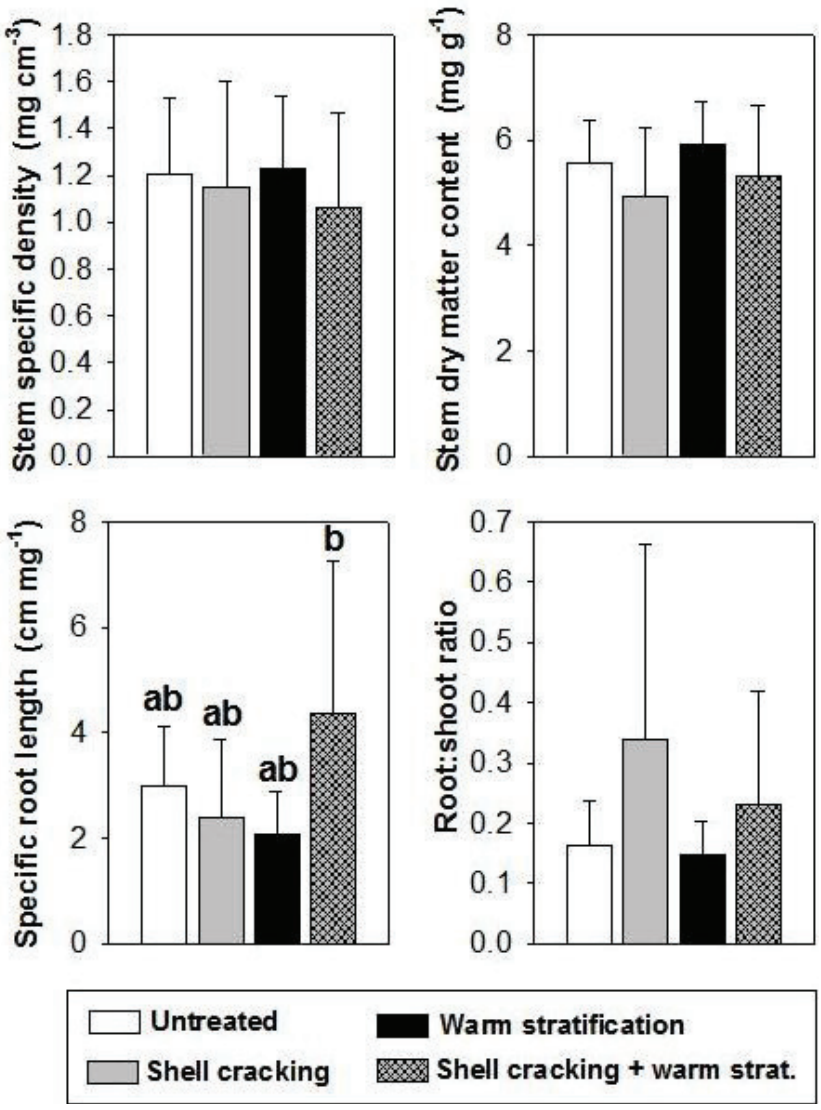

Figure 3. Stem specific density, stem dry matter content, specific root length, and root:shoot ratio of *J. curcas* seedlings growing after different pre-sowing treatments: untreated control, soaking in 30°C water for 24 h, shell cracking, warm stratification at 37°C for 24 h, and shell cracking plus warm stratification at 37°C for 24 h. Values represent means ±S.D. (N=14). When Treatment factor is significant, different letters show significant differences among means (Tukey HDS test, $p \leq 0.05$, N=14).

et al., 2009; Moncaleano-Escandon et al., 2013).

Amongst the tested treatments, warm stratification treatment may be preferred to the other treatments since it expressed a good performance on seed germination and promotes the seedling growth. Pregermination by warm stratification of seeds of tropical plants is common practice of propagation for some important crops like oil palm (*Elaeis guineensis* Jacq.) (Green et al., 2013). The effect of this practice is not only to promote seed hydration but also to enhance the biosynthesis of stress-response substances, like heat-shock proteins, which can play a role in the fast removal of inhibitors of seed germination (Collada et al., 1997; Bailly, 2004; Berjak and Pammenter, 2008). However, the right combination between treatment temperature, time and optimum temperature for germination, in the case of *J. curcas*, should be further investigated. Probably, the treatment temperature at 37°C in our experiment was too high because of the final germination rate inhibition risk, as observed by Windauer et al. (2011) at 35°C. This treatment requires also a certain degree of investment, due to the fact that needed a growth chamber to maintain a constant temperature for a certain period. For this reason, warm stratification treatment could be difficult to

be applied on *J. curcas* seeds collected and processed by farmers in remote areas of developing countries, in which *J. curcas* is generally cultivated at a small scale. Consequently, scarification treatment, allowing a higher root growth, could be a replicable and economic solution to be promoted at small scale.

Further research is needed to both test other different *J. curcas* low-cost pre-sowing treatments, which can be easily practiced in rural areas of developing countries, and monitor the seedling growth for a longer period (> 35 days), in order to collect more data and better evaluate their development. Another aspect to be investigated could be the interaction between seed storage methods and pre-sowing treatments, with the aim to find the optimal combination of these two factors, which could increase both seed germination and seedling growth parameters. In fact, *J. curcas* seed has a relatively short period of viability and high seed storage temperatures, which are common in tropical areas where *J. curcas* grows, and strongly speed up the loss of seed germination (Moncaleano-Escandon et al., 2013).

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Competitiveness of smallholder legume production in South Kivu region, Democratic Republic of Congo

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This study investigated the returns to legumes (common bean and soybean) and other principal crops (cassava, sweet potato and maize) in South Kivu, Eastern Democratic Republic of Congo. Data were collected using a structured questionnaire from a randomly selected sample of 291 farmers who had participated in N2Africa project in the four Eastern D. R. Congo territories: Kabare (103), Kalehe (52), Mwenga (24) and Walungu (112). Gross margin and return on capital analysis were calculated. The study found that common bean had the highest gross margin [985,708 FC ha^{-1} (909FC = 1USD as at June 2013)] and return on labour capital (2.1 FC) compared to other principal crop enterprises. From this study, it was evident that crop enterprises had varying returns on capital which was an indicator of the differences in the importance of these crops from one territory to another. Therefore, the study recommends that as much as legume production is being promoted, the government and NGOs should also emphasize the importance of farm enterprise diversification in the study area.

Key words: Competitiveness, farm enterprises, returns, N2Africa.

INTRODUCTION

More than 30 types of grain legumes are grown across the tropics for food security, income, and improved nutrition and maintaining soil fertility. The most important grain legumes for Sub-Saharan Africa and South Asia are chickpea (*Cicer arietinum)*, common bean (*Phaseolus vulgaris)*, cowpea (*Vigna unguiculata)*, groundnut (*Vigna subterranea)*, pigeon pea (Cajanus cajan) and soybean (Abate et al., 2012). Legumes are important for the livelihoods of millions of rural and urban people throughout the tropics in Africa and East Asia. Legumes provide food and cash; they are also a source of human and animal food. They also provide a positive impact on soil quality which is a major benefit in African farming systems where soils have become exhausted by the need to produce more food per unit of input and where fertilizers are either unavailable or unaffordable for the small-scale producers (Coulibaly et al., 2009). As in other parts of Sub-Saharan Africa, legumes constitute a major part of the population's diet in D.R. Congo. Although there is evidence that D.R. Congo has adequate fertile land for legume production, it is not achieving its potential productivity of 1.6 to 2.0 ton ha^{-1} (Kadima, 2006). In D.R. Congo, legumes are among the main staples consumed. Soybean is extensively cultivated in the eastern region of the country and in the province of Bas-Congo. Soybean cultivation has been encouraged in the Recovery

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Agricultural Plan. Common bean cultivation is practiced throughout the country but good yields are obtained in the upland areas in the east of the country (Orientale Province and in North and South Kivu). In 2007, North and South Kivu provinces produced 70% while the provinces of Bas-Congo and Eastern produced 25% of the total 112,250 tons of beans produced in the country. Bean is a staple crop in the diet of the Congolese population and is commonly found in the market (FAO, 2009). In the light of the importance of grain legumes in D. R. Congo, various nongovernmental stakeholders, such as International Centre for Tropical Agriculture (CIAT) in the N2Africa project, Wageningen University and IITA have come together to address the challenges to grain legumes production and their impact on the livelihoods of the smallholder farmers in developing countries. In regard to these efforts, CIAT is creating an environment for farmers to access inputs such as high yield varieties, rhozobium inoculants, inorganic fertilizers and management information on legume cultivation in Eastern D. R. Congo.

The profitability of legume production using the technology disseminated by N2Africa project has not been compared to other principal crops available to smallholder farmers (cassava, sweet potato and maize) in Eastern D. R. Congo. Therefore, this study investigated the economic returns from the legumes common bean and soybean and the non-legume crops cassava, potato and maize in order to evaluate their competitiveness (ranked importance) and to bridge the existing knowledge gap.

MATERIALS AND METHODS

Study area

This research was undertaken in the Eastern part of DR Congo in South Kivu province, which is located approximately between 1° 36' and 5° South latitude and 26° 47' and 29° 20' east longitude. The province is bounded to the East by the Republic of Rwanda, which is separated by the Ruzizi River and Lake Kivu. In the Southeast, it borders Katanga province; to the south west and northwest Maniema Province and in the North, the North Kivu Province. The province of South Kivu has an area of 69,130 Km^2 and its population was 3,028,000 in1997 and is currently estimated at 3,500,000, with an average density of 50.6 inhabitants per km^2 (Cox, 2008).

The main factors that determine the climate of South Kivu are latitude and altitude. South Kivu province has a mountain climate with mild temperatures (average annual temperature 19°C) with a dry season lasting from 3 to 4 months (June to September) and the rainy season lasts nine months.

During the dry season high temperatures and scarcity of rain is experienced. This is the period when farmers cultivate the swampy areas. The rainy season has high precipitation (Ministry of planning DRC, 2005).

Data collection and sampling design

The sampling frame of the study was made up of small scale legume farmers who were involved in the N2Africa project for at least 2 years in South Kivu. The sampling unit was the farm household. For sampling purposes a multistage sampling technique was employed. In the first and the second stages, purposive sampling was used to select territories and counties. In the third stage systematic random sampling was employed to select the required sample size. Four territories (Kabare, Kalehe, Mwenga and Walungu) and one county in each territory were selected. The sample size of 289 was proportionately determined (Table 1) using the formula by Anderson et al. (2008) as follows:

$$n = \frac{Z^2 pq}{d^2}$$

Where n is the minimum sample size; Z is 1.96 at 95% confidence level; P is the population proportion, that is, the proportion of legume producers in the area. While d is the margin of error (acceptable error) which is assumed to be 0.01 and q is a weighting variable computed as (1-P).

The small scale farmers sample size has been identified using systematic random sampling by dividing the legume farmer's population with the sample size of each territory. Kabare (1328/103), Kalehe (166 /51), Mwenga (192/23) and Walungu (1339/112) indicating sampling was at the interval of 13, 3, 12 and 8 respectively, that is k^{th}+13/3/12/8 from the list provided by CIAT. Data was collected from 300 households to cater for any likely incomplete data; 9 respondents had incomplete data and were dropped thus remaining with 291 which were used for this study.

To achieve the objectives of this study, both primary and secondary data was collected. The primary data was collected through structured questionnaires. The secondary data was collected through the CIAT office in Bukavu from baseline study of N2Africa, office of the minister of agriculture in South Kivu and other related studies to establish the profitability of legume produced traditionally, that is, without the technology disseminated by N2Africa project in D. R. Congo. Secondary data gathered included cost of seeds, labour requirements, output, prices and revenue for common beans and soybean. The use of various sources of secondary data ensured validity and reliability.

Data analysis

Gross margin (GM) analysis was used to assess the economic competitiveness of common bean and soybean using the technology disseminated by N2Africa project compared to those legumes grown without improved technology and compared to cassava, potato and maize produced with locally prevailing technology. The profitability of these crops was determined by calculating the average gross margin, average labour cost, average variable cost, return to labour capital and the return to overall capital for the crops as grown by smallholder households in South Kivu.

The gross margin analysis was estimated by using the formula:

$$GM = \Sigma TR - \Sigma TVC$$

Where; GM = gross margin, ΣTR = total revenue, and ΣTVC = total variable cost.

To bring out the impact of the N2Africa project-disseminated technology, gross margins of legume production using the technology were compared to gross margin of the same without the technology as well as gross margins of competing enterprises (cassava, potatoes and maize).

According to Legesse et al. (2005), farmers engage in production of a certain crop only if the net-returns are higher compared to other alternative crops. Crops often compete for limited inputs and a

Table 1. Population of the territories.

Territories	Total population	Small scale legume farmers population	Sample size
Kabare	496169	1328	103
Kalehe	125141	166	51
Walungu	4456660	1339	112
Mwenga	31747	192	23

Source: CIAT (2011).

Table 2. Competitiveness of legume compared to other principal crops.

Farm enterprises	Average gross margin (FC/ha)	Average labour cost (FC/ha)	Average variable costs (FC/ha)	Returns to labour capital (GM/labour costs)	Returns to overall capital (GM/Variable costs)
Common beans	985,708	467,773	755,640	2.107	1.304
Beans without technology	132,393	162,391	217,607	0.820	0.608
Soy beans	669,869	538,040	629,455	1.245	1.064
Soybean without technology	417,090	558,475	582,910	0.746	0.715
Cassava	203,797	364,744	461,098	0.559	0.442
Potatoes	259,023	145,339	164,663	1.782	1.573
Maize	280,438	188,087	215,358	1.491	1.302

FC, Congolese Franc. 909FC = 1USD as at June 2013.

rational farmer engages in the production of a certain crop only if it remains relatively competitive. Zulu (2011) noted that gross margin analysis appears to be a frequent method used to find out the profitability for different crops in the farming management. Further, Elad and Herbohn (2011) emphasize that farm gross margin provides a simple way for comparing the performance of enterprises. It is also an important and practical tool to indicate farm profit in terms of farm management, budgeting and estimating the likely returns or losses of a particular crop. Similarly, Erbaugh (2008) found that gross margin was a more precise tool to estimate the profitability compared to other budgeting techniques because it includes a determination of costs of each farmer on a per hectare basis on the specific enterprise as well as the revenue earned for each farmer considering the differences in prices. Whereas other techniques such as total revenue or value of farm production include fixed costs of the whole farm, thus tend to overestimate the profit of each enterprise.

Returns to labor and capital for each farm enterprise were used in this study to establish the performance of different farm enterprises. According to Whittaker et al. (1995), partial measures such as; gross margin, budgeting analysis and returns per unit of an input can be used. However, these partial measures do not follow the law of diminishing returns to scale but can be chosen because of their simplicity and flexibility. The returns were estimated by using the formula below:

$$RC = AGM / ALC$$

and

$$R0C = AGM / AVC$$

Where; RC = returns to labour capital, R0C = returns to overall capital, AGM = average gross margin, ALC = average labour cost and AVC= average variable cost.

RESULTS AND DISCUSSION

Average gross margin of legumes (common beans and soybeans) grown with improved technology was higher than legumes or other principal crops grown with prevailing technology (Table 2). However, the average labour cost and the average variable costs were higher for legume production than for other crops. In comparing the various enterprises, the results of the study showed that common beans had the highest returns to labour capital followed by potato, maize and soyabeans and cassava. The return to overall capital was highest for potato followed by maize and common beans. The result showed also that common beans and soybean produced by the technology disseminated by the N2africa project had higher average gross margin, return to labour capital and return to overall capital compared to the legume produced traditionally (without the technology).

For every 1 Congolese Franc (FC) invested in common bean labour there was a return of 2.1 FC (Table 2). For every 1 FC invested in overall capital in common bean production there was a return of 1.3 FC. According to Negash (2007) the gross margins of the improvement technology can be influenced by the accessibility of labour. The farmers with access to a lot of labour are expected to be in a position to try and continue using a potentially profitable new technology and it is expected to influence adoption positively. Choudhary et al. (2011) noted that the gross margin is helpful for the farmer to pinpoint his enterprise issues and improve his specific

Table 3. Profitability of legumes compared to other crops in Kalehe.

Farm enterprises	Average gross margin (FC/ha)	Average labour cost (FC/ha)	Average variable costs (FC/ha)	Returns to labour capital (GM/labour costs)	Returns to overall capital (GM/Variable costs)
Common beans	529824	201504	346254	2.63	1.53
Soybean	770099	328479	346954	2.34	2.22
Cassava	290320	86344	162949	3.36	1.78
Potatoes	182335	71767	72344	2.54	2.52
Maize	85460	15385	16987	5.55	5.03

*FC, Congolese Franc.

Table 4. Profitability of legumes compared to other principal crops in Kabare.

Farm enterprises	Average gross margin (FC/ha)	Average labour cost (FC/ha)	Average variable costs (FC/ha)	Returns to labour capital (GM/labour costs)	Returns to overall capital (GM/Variable costs)
Common beans	1427374	708596	1153039	2.01	1.24
Soybean	609310	546979	621175	1.11	0.98
Cassava	49290	727275	770282	0.07	0.06
Potatoes	164623	268707	353009	0.61	0.47
Maize	571973	194797	250126	2.94	2.29

farm program. The high return to labour capital for common bean production with the new technology shows this crops competitiveness for labour input compared to the other crops studied. However, the return to capital for common bean production was less than potato and about equal to maize indicating this crop is highly competitive for capital but not the highest.

The results of the study also showed that common bean and potato had high return on the overall capital. Nevertheless, potato is not marketable in the study area. Most of the respondents indicated that potato takes a long time to sell in the market and it is not considered as a staple food in the area. According to respondents in the study area, consuming potato 'is not eating'; this means that even after eating potato, people can still eat other foods. Potato meal is not considered a complete meal. According to Kibet et al. (2011), the farmer's profit maximization goal cannot be achieved if the crop chosen is not the most advantageous. Therefore, for farmers to make informed decisions regarding farm enterprise, it is important to understand gross margins of the different crops available to them and the market preference for crops if to be sold as a cash crop.

Common bean production using the traditional technology (without the technology disseminated by N2Africa project) had lower returns on labour and overall capital compared to production using the technology (Table 2) since 1 FC invested resulted to 0.8 and 0.6 FC respectively. Tshering (2012) in a study of profitability analysis of bean production in Honduras found that common beans produced by modern technology had a return of 118 US dollars per hectare while the beans produced traditionally had a return of 70 US dollar per hectare. This is an indicator of the benefits associated with improved production technologies.

When crop production economics are viewed by territory in Kalehe soybean had a high average gross margin as well as average labour cost and average variable cost (Table 3). In comparing the returns to labour capital, maize had the highest return followed by cassava and common beans. Further, the results showed that in comparing the returns to overall capital, maize had the highest return followed by potato and soybean.

In Kabare common beans had the highest average gross margin, while common beans and cassava had the highest average labour cost as well as in average variable cost (Table 4). Maize had the highest returns to labour capital and overall capital followed by common bean and soybean.

In Mwenga (Table 5) soybean had the highest average gross margin, average labour cost and average variable cost followed by maize and common beans. Common beans had the highest return to labour capital and return to overall capital followed by cassava. This could be explained by the low common bean production in these provinces thus supply tends to be lower hence influencing price to be higher. This raised the market value of common bean in this territory and consequently higher return compared to other principal crops. According to the laws of demand and supply, higher

Table 5. Profitability of legumes compare to other principal crops in Mwenga.

Farm enterprises	Average gross margin (FC/ha)	Average labour cost (FC/ha)	Average variable costs (FC/ha)	Returns to labour capital (GM/labour costs)	Returns to overall capital (GM/Variable costs)
Common beans	478296	253863	367086	1.88	1.30
Soybean	1275208	1500324	1648269	0.85	0.77
Cassava	49591	42703	48245	1.16	1.03
Potatoes	57942	59083	73692	0.98	0.79
Maize	503789	777115	809253	0.65	0.62

Table 6. Profitability of legumes compared to other principal crops in Walungu.

Farm enterprises	Average gross margin (FC/ha)	Average labour cost (FC/ha)	Average variable costs (FC/ha)	Returns to labour capital (GM/labour costs)	Returns to overall capital (GM/Variable costs)
Common beans	899923	415766	663508	2.16	1.36
Soybean	549309	420913	549914	1.31	0.99
Cassava	482251	229610	403439	2.10	1.20
Potatoes	281042	84527	53808	3.32	5.22
Maize	54995	135879	148221	0.40	0.37

supply of a commodity leads to a lower price (Ahuja, 2006).

In Walungu (Table 6) common beans, soybeans and cassava had the highest average gross margin as well as the average labour cost and average variable cost. Potato had the highest return to labour capital as well as returns to overall capital followed by common beans and cassava.

According to Chagwiza (2008) the use of gross margin allows the orientation of areas where significant improvement needs to be made in order to optimize production. This is more helpful in the farm management for analysis and planning purposes. However, return to labour measures is important in determining where return on investment could be highest. As a result combining gross margin analysis and return on capital aspects in investment appraissals would boost enterprise selection.

The differences in returns of various crops grown across the four territories (Mwenga, Kabare, Kalehe and Walungu) were a clear indicator of how each of them is important in the livelihoods of the small scale farmers. Farmer crop enterprise diversification ensures compliments in terms of provision of the required nutrients and food security to the households in Eastern D.R. Congo. In addition, diversification of on-farm enterprises reduces production risks associated with agricultural production, for example in case of crop failure. Further, diversification promotes monetary interdependence among the farm enterprises whereby one enterprise can raise capital for initiation of another enterprise or adoption of a new technology. This eases the economic burden on small scale farmers who are mostly resource constrained and have limited access to credit (Msuya et al., 2008; Karani-Gichimu, 2013). It is noteworthy that sometimes farmers do not undertake agricultural activities for profit purposes only but rather for sustenance especially where production can be undertaken without incurring monetary costs by using family labour, household land and low external input technique. This practice is common in Eastern D.R. Congo.

CONCLUSIONS AND RECOMMENDATIONS

From this study, it was evident that different crop enterprises had different returns on capital which was an indicator of the importance of these crops from one territory to another. In the light of this, the study recommends that as much as legume production is being promoted, the government and NGOs should work towards emphasizing the importance of farm enterprise diversification. This would avert the likely effects of legume production failure on small scale farmers.

Conflict of Interest

The authors have not declared any conflict of interest.

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Corn agronomic evaluation under different doses of nitrogen and seed inoculation in savanna

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The agronomic potential of corn is below the genetic potential of high technology hybrid available in Brazil due to the inadequate management of soil fertility, especially of nitrogen fertilizer, essential photosynthetic activity and determining the crop yield. This study evaluated the agronomic performance and yield of maize culture grown with increasing doses of nitrogen (N), in the absence and presence of *Azospirillum brasilense* in brazilian Savanna. The design used was the one of randomized blocks in factorial scheme, with five doses of N in corn (0, 50, 100, 150 and 200 kg ha^{-1}), with and without inoculation of the bacterium, with six replications. It analyzed the N content and leaf chlorophyll was measured plant height, stem diameter, insertion spike length and diameter thousand grain weight, grain rot and productivity. The addition of N fertilizers promotes better agronomic performance of maize plants, being this effect greater in the presence of *Azospirillum*. The productivity of corn respond the dose of 200 kg ha^{-1} N in the presence of *Azospirillum.* In 2012/13 crop corn inoculation with *Azospirillum brasilense* did not cause differences in productivity, plant height, diameter stem, weight of one thousand grains, number of ears and content of chlorophyll A, B and total.

Key words: Fertility, nitrogen fertilization, inoculation, productivity.

INTRODUCTION

The Corn (*Zea mays L.*) is one of the main cereals produced in the world and has as its main producer the United States with approximately 37% of the total, followed by China (22%), Brazil (7.5%), European Economic Community (6.8%) and Argentina (2.8%) (Emygdio et al., 2013). Even occupying the third position in this scenario, with a planted area estimated at 14.2 million hectares and total production of 75.2 million megagrams (Mg) in 2013/2014 season, the national average yield was only of 5.3 Mg ha^{-1}. Those values are lower when compared to the 2012/13 harvest, when the average yield was of 5.4 Mg ha^{-1} and the planted area decreased by around 6.1%, depending on weather conditions and lower use of production technology packages (Conab, 2014), besides the absence of economically viable information that support cultures

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management in tropical conditions (Kappes et al., 2013).

In many regions of Brazil corn production is below the genetic potential of high-tech hybrids, which can reach values ranging from 8.0 to 14.0 Mg ha^{-1}. This low performance has been attributed, among other factors, to the inadequate management of soil fertility through acidity correction, fertilization at planting and coverage, as nitrogen (N) is the nutrient that most limits production because it is essential for plants metabolism. It is also crucial in crop yield, is mainly associated to growth and development of reproductive drains, and participates of the chlorophyll molecule as well. The chlorophyll molecule is essential for the maintenance of photosynthetic activity of plants (Cross et al., 2011). Cantarella (2007) points out that to produce 1.0 Mg of grains, considering a productivity of 12 Mg ha^{-1} and 7 kg ha^{-1} of N are needed.

The macro and micronutrients are usually provided via mineral fertilizer of high solubility, however, the intensive use of inorganic fertilizers can cause nutritional imbalance of plants and influence on the quality of the final product. As for N, one of the alternatives used to reduce consumption of these fertilizers is the biological N fixation (BNF) held by N-fixing bacteria in association with the plant. That is already being successfully used in corn (Lana et al., 2012; Sabundjian et al., 2013), rice (Puente et al., 2013) and cane sugar (Pereira et al., 2013). Specifically for corn, *Azospirillum brasilense* is a bacterium that has provided positive results, once besides producing hormones for the plants, it is also capable to fix atmospheric N. When inoculated in corn it becomes a promising technique for the plants development and increased productivity.

Currently there are technological packages using varieties of plants and efficient bacterial strains. They can supply more than 50% of the N required for the plant (Ferreira et al., 2013). Yet the growth stage of the plant is a determining factor for coverage with N, once it is recommended to apply the remaining amount of the nutrient in whole or split dose what must be made between stages (V4) and (V8) of fully expanded leaves (Fancelli, 2010).

The definition of the number of rows and the size of the ear, which are components of the corn grain yield, is between stages V4 and V12. These stages occur between 40 and 60 days after emergence, a period in which there is the most intense absorption of the nutrient, which is the ideal time to assess the nutritional status of the plant. Rambo et al. (2004) emphasize that the analysis of chlorophyll content is an important parameter for the assessment of plants development, widely used to differentiate plants with (in) adequate levels of N.

The inoculation of maize seed with *Azospirillum* bacteria has proven to have high potential for reducing N fertilization. It improves the physiological characteristics of the plant and can provide increased crop productivity as well (Goês et al., 2013). However, the influence of that association on the macro and micronutrients accumulation in the plant in stages that precede the definition of the crop yield need better assessment, as they have been provided for the most part in the planting via mineral fertilizer. Given this context, this study evaluated the agronomic performance and corn yield grown with increasing doses of N, in the absence and presence of *Azospirillum brasilense* in Brazilian Savanna.

MATERIALS AND METHODS

The study was conducted in the area of the Experimental Farm Capim Branco, between geographic coordinates 18° 55'23" S and 48°17'19" W, at an altitude of 872 m, during the two crops cycles (2011/12 and 2012/13). The area belongs to the Federal University of Uberlândia (UFU), in Uberlândia-Brazil.

The climate is classified as Aw, tropical with a dry season in winter, according to the Köppen classification. Annual average precipitation in the region is up to 1500 mm. The soil was classified as Red Latosol, clayey in texture, containing na arable layer (0 to 0.20 m), 580 g kg^{-1} clay, pH H_2O = 5.5; 2.9 mg dm^{-3} P; 101.0 mg dm^{-3} K; 12.0 mg dm^{-3} S; 1.0 $cmol_c$ dm^{-3} Ca; 0.5 $cmol_c$ dm^{-3} Mg; 0.0 cmolc dm^{-3} Al; 0.11 mg dm^{-3} B; 2.6 mg dm^{-3} Cu; 9.0 mg dm^{-3} Fe; 0.7 mg dm^{-3} Mn; 0.3 mg dm^{-3} Zn; 21 g kg^{-1} organic carbon; 4.86 $cmol_c$ cm^{-3} cation exchange capacity at pH 7.0 and 36% bases saturation.

The experimental design was a randomized block in a factorial 5 × 2, with five N in corn (0, 50, 100, 150 and 200 kg ha^{-1}), two forms of inoculation of the bacterium: (1) Inoculated with *Azospirillum brasilense* with a dose of 100 mL ha^{-1}, with a minimum concentration of 2×108 cells ml^{-1}. (2) No inoculation, with six replications.

The area was prepared for planting with broadcast application of 1.0 Mg ha^{-1} of dolomitic limestone, 40.2% CaO and 14% MgO, relative neutralization total power (PRNT) 100% built with heavy harrowing, followed by the application of 1,0 Mg ha^{-1} of gypsum built with heavy harrowing. Then we used leveling grating and scarifier to open the grooves, with seeding being done manually on 14/12/2011 and 23/11/2012, using 3.5 seeds per meter, to obtain the stand of 70.000 plants per hectare.

The corn hybrid used was DKB 390 VTPRO and the inoculant was the commercial product Masterfix Gramínea® (strains - AbV5 and AbV6). Each plot consisted of 10 lines with 6 m long, spaced 0.5 m and the plot useful for harvest consisted by four core lines, excluding one meter from each end.

The planting of corn in the year 2011/12 was applied by 18 kg ha^{-1} for Mg and 24 kg ha^{-1} S in the form of magnesium sulphate (9% Mg and 12% S); 120 kg ha^{-1} P_2O_5 as triple superphosphate; 50 kg ha^{-1} K in the form of potassium chloride (KCl) and 50 kg ha^{-1} of N in the form of urea (44% N), except for the treatment with doses of zero N. The fertilization of covering held in stage of development V4 consisted of the application of 100 kg ha^{-1} K as potassium chloride and N dose required to complete the dose of each treatment. In the stage V8, foliar fertilization was performed with 40 g ha^{-1} molybdenum; 4.0 g ha^{-1} cobalt; 300 g ha^{-1} manganese and 147 g ha^{-1} of sulfur, 400 g ha^{-1} boron and 2 kg ha^{-1} zinc through ground.

While the planting of corn in the year 2012/13 was applied 120 kg ha^{-1} P_2O_5 as triple superphosphate 50 kg ha^{-1} K in the form of potassium chloride and 50 kg ha^{-1} of N as urea, except in the treatments with doses of zero N. The fertilization of covering held in stage of development V4 consisted of the application of 100 kg ha^{-1} K_2O as potassium chloride and N dose required to complete the dose of each treatment. Fertilization was performed 300 g ha^{-1} of boron with tetrahydrated sodium octaborate (20% B) via soil and 2 kg ha^{-1} zinc with zinc oxide (76% Zn) via foliar.

In stage V7 we performed a weeds control using backpack

Table 1. Chlorophyll content A, B and Total related to N doses, in the absence and presence of bacterium *Azospirillum brasilense*, corn stage V8 in 2011/12 and 2012/13 crops, Brazil.

Bacterium	N doses									
	2011/12					2012/13				
	0	50	100	150	200	0	50	100	150	200
	kg ha^{-1}									
	Chlorophyll A									
Absence	33.6^{Aa}	34.3^{Aa}	35,9^{Aa}	36,0^{Aa}	36,2^{Aa}	35,3^{Aa}	38,2^{Aans}	38,7^{Aa}	38,0^{Aa}	38,9^{Aa}
Presence	35.2^{Aa}	34.8^{Aa}	36,4^{Aa}	36,0^{Aa}	36,8^{Aa}	35,7^{Aa}	40,0^{Aa}	39,2^{Aa}	39,1^{Aa}	39,2^{Aa}
CV %			4.55					4.57		
	Chlorophyll B									
Absence	14.5^{Aa}	15.8^{Aa}	17.8^{Aa}	18.3^{Aa}	19.3^{Aa}	14.4^{Aa}	19.3^{Aa*}	20.7^{Aa}	20.4^{Aa}	21.4^{Aa}
Presence	16.1^{Aa}	16.5^{Aa}	19.0^{Aa}	18.7^{Aa}	20.3^{Aa}	14.9^{Aa}	22.7B^{a}	21.5^{Aa}	20.8^{Aa}	21.5^{Aa}
CV %			13.61					12.85		
	Tot^{a}l chlorophyll									
Absence	48.2^{Aa}	50.1^{Aa}	53.7^{Aa}	54.3^{Aa}	55.6^{Aa}	49.8^{Aa}	57.6^{Aa}	59.5^{Aa}	58.4^{Aa}	60.4^{Aa}
Presence	51.3^{Aa}	51.2^{Aa}	55.4^{Aa}	54.8^{Aa}	57.1^{Aa}	50.7^{Aa}	62.8B^{a}	60.7^{Aa}	59.9^{Aa}	60.7^{Aa}
CV %			7.45					7.12		

* = Significant ($p < 0.05$); ns = Not significant. Means followed by the same capital letter comparing presence and absence in the dose evaluated in the column and minuscule line in comparing the two years in the same dose, do not differ by Tukey test ($p < 0.05$).

sprayer with spray, volume of 350 L ha^{-1}. Herbicides used were: Atrazine (400 g L^{-1}) dose of 4.0 L ha^{-1} and tembotrione (420 g L^{-1}) at a dose of 0.24 L ha^{-1} with Aureo adjuvant (methyl ester soybean oil - 720 g L^{-1}) at a dose of 0.35 L ha^{-1}.

Plants were harvested in May 2012 and April 2013, with the grain moisture corrected to 13% for productivity calculation. In the two years (2011/12 and 2012/13) we evaluated:

1. The chlorophyll content (A, B and total) on the stage V8 using chlorophyll ClorofiLOG® model CFL1030.
2. The leaf N in stage V8, using the last fully developed leaf in the stadium R1 and R3 (spike leaf in the reproductive phase), according to the methodology proposed by Embrapa (2009).
3. The spike length and diameter, which were measured with a graduated ruler (cm) and pachymeter (mm), weight of thousand grains, percentage of burning grains from a sample of 250 g grain from of each plot.
4. Fresh and dry weight will be evaluated in handy when the plant reaches flowering.
5. Productivity, obtained from the weighing of grain harvested in the useful portion, adjusted the humidity to 13%.

The results were analyzed for normality and homogeneity of data through tests Lilliefors, Cochran and Barttlet. The results were submitted to variance analysis, and applied the F test for significance and means compared by Tukey test ($p < 0.05$) for the variable inoculation using Sisvar software version 4.0, being made a regression analysis to dose Sigmaplot using the 2010 version.

RESULTS AND DISCUSSION

Analyzing the effect of increasing doses of N on the parameters evaluated in 2011/2012 and 2012/2013, it was observed that the value was significantly higher in the presence of bacterium Azospirillum brasilense only in the dose of 50 kg ha^{-1} in the year 2012/13 (Table 1), showing that the higher N absorption increased the chlorophyll content in the leaf, which implies a higher rate of photosynthesis, thus in greater carbon accumulation in the plant. This biomass accumulation, associated to increased absorption of water and nutrients, increases the translocation and accumulation of nutrients to the grains, which demonstrates the possibility of using these bacteria as promoters of plants growth.

Similar results were observed in other studies with seed inoculation by bacterium *Azospirillum brasilense*, which had higher chlorophyll content and growth in cane sugar (Pereira et al., 2013) and corn (Kappes et al., 2013). However, for corn results are still divergent, as Morais (2012) has made the seed inoculation with the same bacteria observing no changes in leaf chlorophyll content.

Increasing N levels in the presence and absence of *Azospirillum* promoted linear response of chlorophyll content A, B and total in 2011/12 harvest. In the next harvest an increase in chlorophyll contents A, B and total was observed according to increasing doses of N, up to doses of 122, 153 and 142 kg ha^{-1} N, respectively, providing quadratic increase in leaf chlorophyll content (Figure 1). Above these doses, there was reduction in chlorophyll content, due to the plant no longer respond to the increased supply of N.

According to Rambo et al. (2004) the relative chlorophyll content in the leaf has been considered the best indication of the N level and according to Malavolta

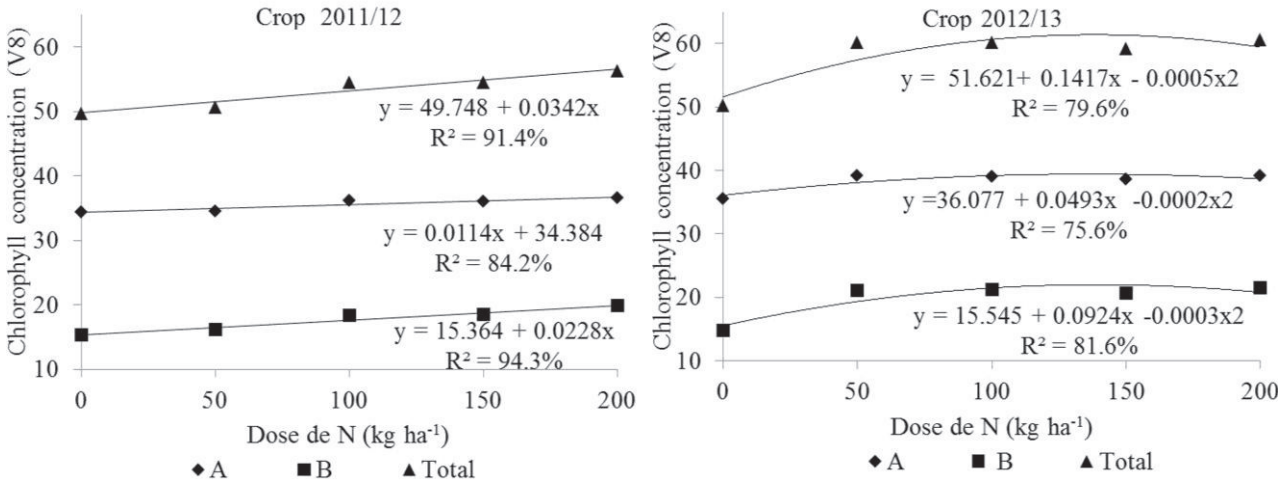

Figure 1. Contents of chlorophyll (A, B and Total) due to increasing doses of N in the stage V8 of corn coverage, Brazil, 2011/12 and 2012/13 seasons.

et al. (1997 that is due to its practicality and economy of the determination by chlorophyll meters. Furthermore, the determination of the relative content of chlorophyll has been used to predict the need for N fertilization in coverage in various crops, among the corn (Argenta et al., 2004).

With respect to N uptake by the corn crop, the maximum absorption occurs in two distinct periods, in the stages of vegetative and reproductive development or formation of the corn ear, while the lowest absorption rates occur in the period between the issuance of the tassel and the beginning of the formation of the ear (Olness and Benoit, 1992).

For foliar N content in the V8 stage, R1 and R3 in the years 2011/12 and 2012/2013 there was no significant increase in values was applied when the evaluated doses, however, in general, all observed values of this parameter were within the sufficiency range (28 to 35 g kg^{-1} of N), considered suitable for corn (Malavolta et al., 1997).

Similar results were found by Gôes et al. (2013) and Kappes et al. (2013) for corn and by Pereira et al. (2013) for cane sugar. However this study also points to the specificity between bacteria and genotypes, since this response was not found in other varieties evaluated. That was also highlighted by Dotto et al. (2010) who found no significant difference in leaf N content due to N levels or inoculation. However, leaf N content in the hybrid AS 1540 was higher if compared to hybrid 1570, demonstrating the greater efficiency in AS 1540 N absorption. These results point to the need for further research involving the bacteria-genotype relationship, since the different materials have particular genetic characteristics and, therefore, the interaction with the bacteria can occur differently.

In the growth stages R1 and R3 there were no significant interactions among the different doses of N and the presence or absence of *A. brasilense* inoculation. Through regression analysis shown in Figure 2 of 2011/12 season, there is a linear increase due to the doses applied, because the higher the amount of N applied, the greater the foliar N content, for stages V8 and R1. In stage V8 an absorption increase of this element is verified because the roots have a strong development (Fancelli, 2010). For each 1.0 kg ha^{-1} of N applied, there is an increase of 0.0171 g kg^{-1} of foliar N to stage V8, with good predictive capacity of the 88.57% model.

In the R1 stage, with the maximum dose of 200 kg N has 30.98 g kg^{-1} of N leaf, which every 1 kg of N applied, the increase in leaf N content in the R1 stage is 0.0163 with predictive ability of the model 91.62%. For stage R3 there was an increase in foliar N content as doses went up to 129 kg ha^{-1} N, where the foliar content was of 29.41 g kg^{-1}. Goês et al. (2013) found a quadratic response of foliar N content by applying increasing doses of urea in corn.

Through the regression analysis shown in Figure 2 of 2012/13 crop, it is observed that according to the equation obtained in stage V8, with the highest dose applied of N in this experiment (200 kg ha^{-1}), it would be possible to achieve maximum N content in leaves (40.41 g kg^{-1}). For Cruz et al. (2011) there is a major impact of the N metabolism in the growth and yield of corn, because this nutrient establishes and maintains the photosynthetic capacity. Therefore, in order to achieve high yields one has to keep this activity during seed formation and grain filling.

Analyzing the average values of agronomic parameters evaluated in 2011/2012, it was observed that there was an increase in plant height and the height of ear insertion depending on the dose of 200 kg ha^{-1} of N in the

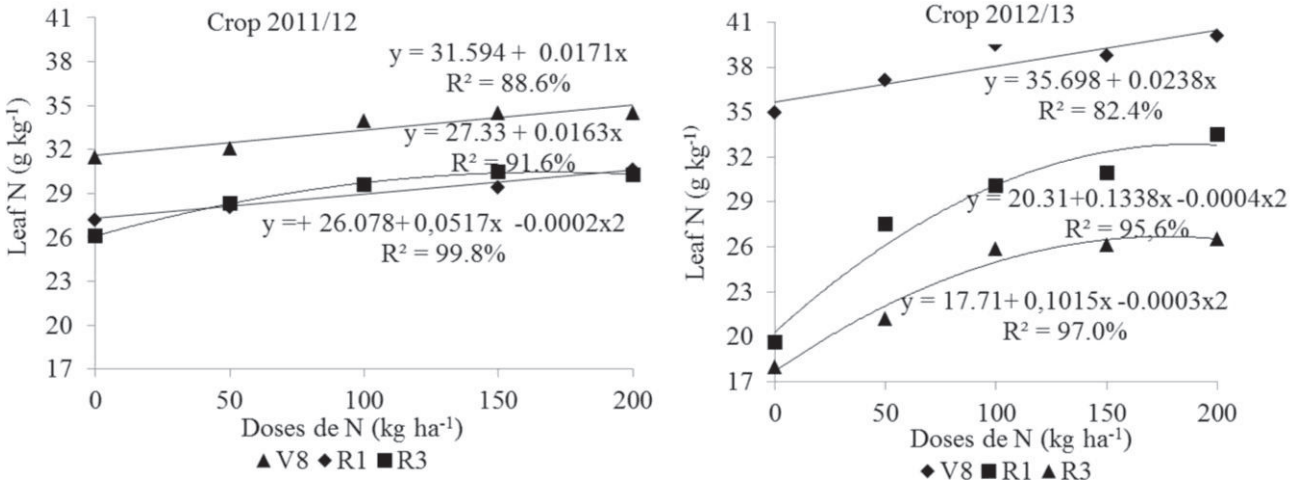

Figure 2. Content of foliar N due to increasing doses of N, in stages V8, R1 and R3 of corn crop harvests in 2011/12 and 2012/13, Brazil.

presence of the bacterium, while the same was not true for the year 2012/2013.For a stem diameter there was no influence of doses of N or seed inoculum evaluated in the two years (2011/2012 and 2012/2013) (Table 2). These results show that high doses of N associated with seed inoculation with Azospirillum alter the physiological response of maize, demonstrating the importance of N in plant metabolism because it is essential to the biosynthesis of amino acids, proteins, chlorophyll, hormones (Rambo et al., 2004).

For plant height parameter Lana et al. (2012) found no significant difference according to the inoculation with *Azospirillum* spp. or N fertilization. With regard to the ear insertion height, similar results were observed by Goês et al. (2013), who found a quadratic response to this insertion height as a function of N doses, both as urea and ammonium sulphate applied to corn in winter cover.

Evaluating N fertilization and inoculation with *A. brasilense* and *Herbaspirilum seropedicae* in corn Dartora et al. (2013) obtained 15% increase in stem diameter in the treatments inoculated with respect to the control treatment, while Dotto et al. (2010) observed divergent results because they did not find significant effect of dose or inoculation on the stem diameter.

Among evidenced average values of fresh and dry weight of the plant (leaves + stem) it was observed that these indices have increased linear response to N rates evaluated in the presence and absence of Azospirillum (Figure 3).

In 2011/2012 in the presence of *Azospirillum*, fresh weight increase was of 1.08 g kg^{-1} of N, therefore, higher than in its absence when the gain was of only 0.67 g kg^{-1} of N. In the dry mass, the increase was of 178 mg kg^{-1} N. These differences are due to the ability of these bacteria to synthesize plant hormones that once available to plants, stimulate the growth of fine roots of plants, thus increasing their ability absorption of water and nutrients (Oliveira et al., 2009).

The plant dry matter is important because it sets the amount of carbohydrates to be translocated to the grain, so it is directly related to the size of grains as well as the production of the plant. According to Sangoi et al. (2005) the dry weight response in the presence of *Azospirillum* may be related to the ability of the bacterium to provide part of the fixed N_2 to the plant, because N is absorbed in large amounts by corn. As a constituent of amino acids, proteins, chlorophyll, its greater availability for culture can promote increases in carbohydrate accumulation by the plant.

In 2011/2012 season ear length was not affected by the application of increasing doses of N or seed inoculation, while the diameter increased linearly with increased N doses up to 200 kg ha^{-1} (Figure 3). Seed inoculation increased the diameter in 23%. That difference is probably due to the improved root system generated by *Azospirillum*, increasing nutrient uptake by the plant, resulting in larger ear diameter.

Goês et al. (2013) evaluated sources and doses of N in winter cover, and Okumura et al. (2013) evaluated doses of urea treated with urease inhibitor. They have found respectively quadratic and linear adjustment of the ear diameter according to the increasing doses of N in corn.

In 2011/2012 and 2012/2013 the incidence of rotted grains in this study was not significantly influenced by the application of different doses of N, as they are normally resulting from the decay of eyes due to fungi present in the production field (*Fusarium* spp., *Gibberella zeae*), which occurs mainly when the grains remain in the field after physiological maturity until harvest (Marcos, 2005). However, if we compare only the averages in the dose of 200 kg ha^{-1} N, the percentage of rotten grains is 44% lower in the presence of *Azospirillum* (Figure 3).

Table 2. Plant height, ear height, stem diameter, fresh and dry matter (leaves + stems) depending on N doses, with and without seed inoculation with *Azospirillum brasilense* in 2011/12 and 2012/13 seasons, Brazil.

Bacterium	N doses									
	2011/12					2012/13				
	0	50	100	150	200	0	50	100	150	200
	Plant height (m)									
Absence	2.08^{Aa}	2.10^{Aa}	2.16^{Aa}	2.12^{Aa}	2.09^{Ba*}	2.05^{Aa}	2.12^{Aa}	2.17^{Aa}	2.13^{Aa}	2.18^{Aa}
Presence	2.07^{Aa}	2.09^{Aa}	2.16^{Aa}	2.14^{Aa}	2.17^{Aa}	2.06^{Aa}	2.13^{Aa}	2.19^{Aa}	2.16^{Aa}	2.22^{Aa}
CV %			3.45					2.95		
	Ear insertion height (m)									
Absence	1.20^{Aa}	1.22^{Aa}	1.23^{Aa}	1.22^{Aa}	1.18^{Ba}	1.09^{Aa}	1.12^{Aa}	1.15^{Aa}	1.14^{Aa}	1.16^{Aa}
Presence	1.19^{Aa}	1.20^{Aa}	1.24^{Aa}	1.21^{Aa}	1.25^{Aa}	1.09^{Aa}	1.14^{Aa}	1.18^{Aa}	1.15^{Aa}	1.21^{Aa}
CV %			4.50					5.07		
	Steam diameter (mm)									
Absence	21.20^{Aa}	21.4^{Aa}	21.8^{Aa}	23.2^{Aa}	2.8^{Aans}	20.18^{Aa}	21.6^{Aa}	22.8^{Aa}	22.4^{Aa}	22.9^{Aa}
Presence	21.10^{Aa}	21.5^{Aa}	21.6^{Aa}	22.5^{Aa}	22.3^{Aa}	20.78^{Aa}	21.9^{Aa}	23.9^{Aa}	22.8^{Aa}	24.0^{Aa}
CV %			17.64					11.75		

* = Significant (p <0.05); ns = Not significant. Means followed by the same capital letter comparing presence and absence in the dose evaluated in the column and minuscule line in comparing the two years in the same dose, do not differ by Tukey test (p <0.05).

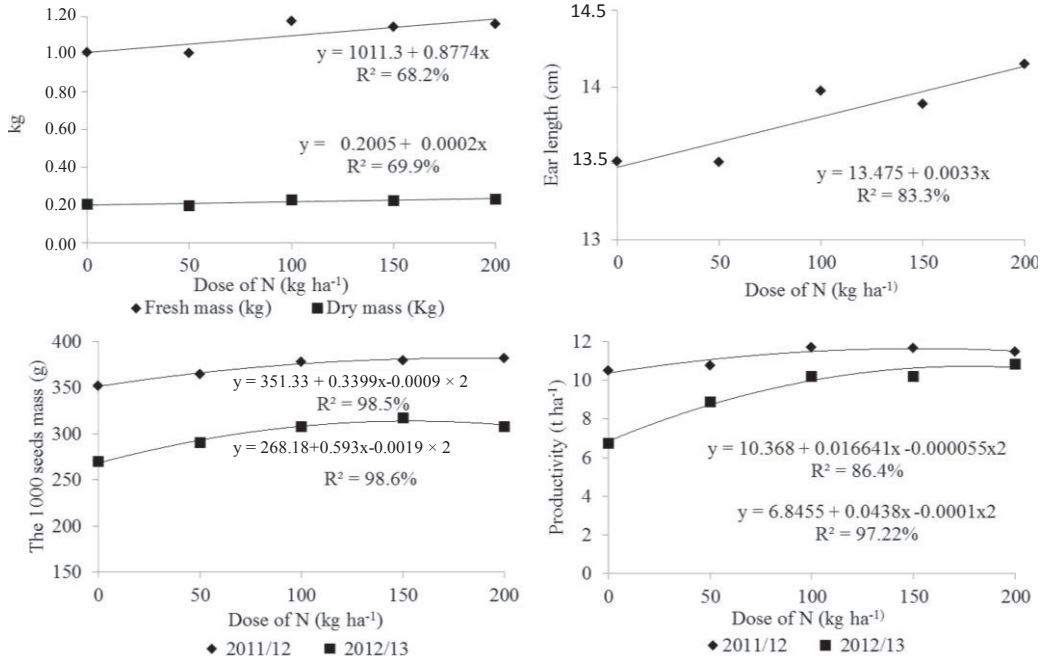

Figure 3. Fresh and dry mass (kg), ear length (cm), weight of 1000 grains (g) and yield (Mg ha^{-1}) as a function of increasing doses of N, in corn, 2011/12 and 2012/13 crops, in Uberlândia, Brazil.

As for the thousands grain weight in 2011/2012 harvest a smaller grain size was observed in the presence of *Azospirillum* and absence of N since the weight of a thousand grains was smaller. However, with increasing levels of N, the increment in the presence of the bacterium was of 200 mg kg^{-1} of fertilizer applied to corn, whereas in the absence of the bacterium this increase was of 100 mg kg^{-1}, that is, twice when the fertilizer N was associated to inoculation (Figure 3). In 2012/2013 there was no significant difference for this parameter, depending on the absence or presence of *A. brasilense.* According to Borrás and Otegui (2001) the production

component was the one less affected by changes in management and fertilization practices.

When studying the management of N in coverage in maize grown under no-tillage Souza and Soratto (2006) did not observe any significant effect on the culture grain mass in conformity with what was observed in this study. Kappes et al. (2013) also obtained similar results by testing application methods and *Azospirillum* doses in corn, also finding no significant difference in the thousands grain weight.In this study corn hybrids used had a high productive potential and responded to high doses of N.

In 2011/2012 there was increased productivity to the highest dose tested (200 kg ha^{-1}) in the presence of *Azospirillum* in which the estimated yield was of 12.0 Mg ha^{-1}. This may be associated to *Azospirillum* which increases the amount of fine roots, enhancing the absorption and accumulation of nutrients by plants (Bashan and de-Bashan, 2010). In the absence of the bacterium, when N dose was increased from 0 to 200 kg ha^{-1} there was an increase of 3% (314 kg) in grain production, while in presence of the bacterium the increase was of 16% (1630 kg) (Figure 3).

In 2012/2013 there was significant interaction among the N doses applied because corn yield is strongly influenced by the availability of N in soil, as evidenced by other studies (Santos et al., 2010), but there was no significant effect between seed yield with inoculation (9.31 Mg ha^{-1}) and without inoculation (9.43 Mg ha^{-1}), what gives the results obtained by Dotto et al. (2010). Despite that, around 160 bags per hectare were obtained. In 2012/13 quadratic response of grain yield was found as a function of N doses, with maximum productivity (13.1 Mg ha^{-1}) achieved in the application of 200 kg ha^{-1} of N. The corn production was 60.9% higher with increasing N doses (Figure 3), if compared to the control.

Bartchechen et al. (2010) found in a study of commercial inoculant based on *A. brasilense* associated to different doses of N that inoculation yielded superior results on maize productivity in relation to the witness, but there were no differences in productivity with isolated or associated inoculation to N topdressing. Morais (2012) found quadratic response of grain yield in tests with high technology in corn as a function of N doses, being the maximum productivity (10 to 14 Mg ha^{-1}) achieved in the application of 260 kg ha^{-1} of N.

Conclusion

The addition of N fertilizers promotes further development of corn plants, increases the levels of chlorophyll and nutrients, plant biomass, ear diameter, thousands grain weight and productivity, which has greater effect in the presence of *A. brasilense*. The inoculation of maize seeds with *A. brasilense* improves absorption efficiency of N. The productivity of maize grains responds to the dose of 200 kg ha^{-1} of N in the presence of *A. brasilense*. The inoculation with *A. brasilense* does not replace the use of N fertilizers, but improves plant response to fertilization, especially in high doses. Corn production and chlorophyll levels A, B and total were significant with increasing N doses. In 2012/13 corn seed inoculation with *A. brasilense* did not provoke significant differences in productivity, plant height, stem diameter, thousands grain weight, number of ears and content of chlorophyll A, B and total.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Fungal population, including Ochratoxin A producing *Aspergillus* section *Nigri* strains from Ivory Coast coffee bean

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The mycotoxin contamination in foods has become a growing threat and aroused many researches in science area. According to the food control authorities, Ochratoxin A (OTA) is among mycotoxins priority of food contaminants. The current work focuses on coffee cherries quality environment in Ivory Coast. The neglecting of good agricultural practices would lead to recurrent contamination of ivorian coffee in mycotoxinogen fungi and mycotoxin. We investigated and sampled during the post-harvest drying process of *Robusta* coffee bean, brought from Ivory Coast in 2008 and 2009. Morphological identification of total fungal flora and the determination of OTA producers of *Aspergillus* section *Nigri* have been performed. The reliability of the overall evaluated fungal contamination is estimated at 97%, being 7.03 in one coffee sample package of 300 g. Strains were isolated on potato dextrose agar (PDA) by the direct plating technique, and were grown at 25°C. Morphological study was performed using macroscopic and microscopic morphological characters. From the two hundred and eighteen strains of fungi isolated, the following were identified: *Aspergillus* section *Nigri*, *Aspergillus* section *Fumigati, Penicillium*, *Mucor, Fusarium* amongst others. *Aspergillus* section *Nigri* was found to be the most important group representing 52% of the population. Within this section OTA production was evaluated on czapeck yeast agar (CYA) and quantify by High-performance liquid chromatography (HPLC). Twenty percent of produced detectable OTA with concentrations ranging from 0.3 to 56 µg/g of agar medium. The objectives of this study is to define the risk contamination in post-harvest fungi on coffee.

Key words: Agriculture, coffee, process, filamentous fungi, mycelium, *Aspergillus*, mycotoxin, Ochratoxin A.

INTRODUCTION

Coffee is very valuable. However, it is subjected to various pest and diseases of which mycotoxin contamination is of great importance in terms of the health of consumers and economic loss (Paterson et al., 2014). Fungal contamination of coffee has been reported from all over the world. Many researchers have worked

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on coffee microbiology process (Silva et al., 2000; Pandey et al., 2000; Noonim et al., 2008; Nakajima et al., 1997; FAO, 2006a) and show that microorganisms are naturally present in all pre-and post coffee harvests and might influence coffee quality. Microbial biodiversity present in coffee cherries and beans depends on the coffee variety, processing method, and environmental factors of the region in which they are cultivated (Bucheli and Tanikawa 2002; Batista et al., 2009). Two methods are carried out for the post-harvest process of coffee: dry and wet methods (Schwan and Wheals, 2003). Therefore the impact of microbial and their distribution vary from one treatment to another. From East to West area, post-harvest and cultural practices differ according to the income of farmers. Throughout coffee area in Côte d'Ivoire, post-harvest treatments have been improved. In the some localities where the farmers suffer the pangs of more tradition and are threatened constantly by poverty, post-harvest treatments are more rudimentary. Generally ripped cherries are harvested in one go and dried on traditional media. Then they are peeled by machine or pounded in a mortar and stored in bags of raffia, at the farmer or in storage. After shelling, coffee cherries undergo a process of natural drying on different support like rack, plastic film, ground, asphalt and cement. The type of drying is free and depends on the income of the farmer. Tropical moisture and storage conditions sometimes expose cherries and coffee beans at a constant postharvest mold contamination. Some studies on coffee (Kouadio et al., 2010, 2012), report that fungi are constantly present on coffee samples from Côte d'Ivoire, whatever their type and conditions of post-harvest treatment. Endogenous mycobiota of coffee cherries, coming from coffee cherries ecosystem and various contaminations which it is subjected during steps of washing cycle (more or less microorganisms in water), drying, transport and storage are also potential sources of contamination in filamentous fungi. Mycobiota involved in coffee fermentation or contamination come from the commensally flora of the cherries, soil, washing water and equipment used. The microbial load depends on physico-chemical parameters (temperature, pH, Aw), the composition of the substrate in simple sugars and/or polysaccharides, but also for the maintenance of equipment used, drying areas, storage and warehouses quality. Research reports that moisture pulp and its mucilaginous material (regarding its biochemical composition) promotes development of this endogenous mycobiota (Avallone, 2001).

Several mycotoxigenic strains of *Aspergillus* section *Nigri* have been isolated on Robusta coffee in Côte d'Ivoire (Kouadio et al., 2012), in Brazil (Taniwaki et al., 2003), in Thailand (Noonim et al. 2008; Joosten et al., 2001) and Vietnam (Leong et al., 2007).Within them *Aspergillus* spp. like *Aspergillus carbonarius*, *Aspergillus niger*, *Aspergillus terreus* in section *Nigri* and *Aspergillus versicolor, Aspergillus ochraceus, Aspergillus alliaceus* (Bayman et al., 2002) in other section have been recognized as Ochratoxin A producers in Wheat (Riba et al., 2008), in cocoa beans (Sanchez-Hervas et al., 2008), in robusta coffee beans (Kouadio et al., 2012), in peanuts seed (Magnoli et al., 2007), in grapes (Salma et al., 2007) and olive fruits (Roussos et al., 2006). Other strains as *Aspergillus sclerotioniger* and *Aspergillus lacticoffeatus* are rarely OTA producing strains. Contamination of food commodities, including cereals and cereals products, nuts and species with OTA has been reported from all over the world. Mycotoxins have been ranked as ''the most important chronic dietary risk factor, higher than synthetic contaminants, plant toxins, food additives, or pesticide residues'' (Kuiper-Goodman, 1998; Bennet and Klich, 2003). In fact food contamination by mycotoxins has been recognized as a public health threat (JECFA, 2002). Ochratoxin A as well as the Aflatoxins B1and M1and the Deoxynivalenol (DON) have been classified in the four priority mycotoxins of food contaminants by the Global Environment Monitoring System/Food Contamination Monitoring Assessment Programme (GEMS/Food) of the WHO (2002). Ochratoxin A mycotoxin produced by fungi of the genera *Aspergillus* and *Penicillium*, is a food contaminant found naturally in various agricultural products such as grains, oil seeds, nuts, coffee and cocoa (EU, 2010; EFSA, 2006; CCA, 2007). OTA is a critical element in the quality of food because it is thermostable and can be found in the finished products from agricultural raw materials contaminated even after industrial processing well done. Indeed, this may be a great loss if a food highly contaminated with OTA is declared unsuited for human and/or animal consumption (Dano Djédjé et al., 2009). Chemically Ochratoxin A, belong to a group of fungal metabolites that have a wide variety of toxic effects. It has been listed as possibly carcinogenic to humans (group 2B) by the International Agency for Research on Cancer (IARC) (Castegnaro et al., 2006). It causes renal toxicity, nephropathy and immuno-suppressive in several animal species, resulting in reduced performance parameters in animal production. OTA has also been detected in blood and other animal tissues and in milk, and has been implicated in the fatal human diseases Balkan Endemic nephropathy (Marquardt and Frohlich, 1992). Since it has been discovered several analytical methods already formally validated in team-work such as qualitative and quantitative methods are available. The particularity of qualitative techniques as ELISA kits rapid detection or Immune Radio Essay (RIA) is the fact that they require little purification, whereas techniques of quantitative detection require analysis method including a more complex purification-extraction phase and an analytical procedure (Berger et al., 1999). For filamentous fungi, technique qualitative detection of Ochratoxin A consists in demonstrating toxin production after growth of filamentous fungi on a specific agar to 60% of coconut cream. The visualization of fungal colony

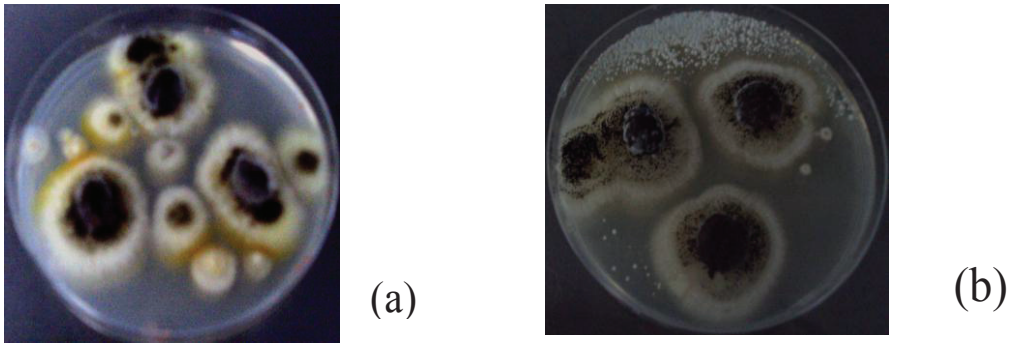


Figure 1. Colonies of filamentous fungi on coffee cherries by direct contact technique on PDA agar, 25°C (3days).

under UV light shows a blue-violet fluorescence around the strain tested if it is Ochratoxin A producer. As for quantitative techniques, they are modelled on analytical methods accepted by chemists internationally (AOAC, 2000; Raquel Duarte et al., 2008). These methods are constantly being updated in line with technological progress (CCA, 2007). The process of studying the ochratoxinogen potential of filamentous fungi require an extraction step focus on various media: solid synthetic media (czapeck yeast agar) (Bragulat et al., 2001) or liquid media (yeast extract sucrose broth, potato dextrose broth) (Bayman et al., 2002), and natural carbon substrates such as rice according to the official FDA method (Tournas et al., 2001), the coffee bean (Pitt, 2000). It has followed by a purification step which using methods are based on the specific nature of the solvent for OTA and its ability to releasing all interfering compounds OTA. Analytical procedures for mycotoxins determination have been improved continuously over the past years. Chromatographic methods have been used widely, including thin-layer chromatography (TLC) (Pittet and Royer, 2002), gas chromatography (GC) with electron capture detection (ECD) or mass selective detection (MS) as well as high-performance liquid chromatography (HPLC) with UV or fluorescence detection, also with (multiple) mass spectrometry as described in more recent publications (Berger et al., 1999). It should be noticed that there is no preset method of analysis because the analytical procedures and purification extraction methods are multiple and closely linked to the nature of the extraction matrix of OTA and OTA material analysis (solvent extraction, purification kit, quantification and detection devices) which represent a significant financial cost. For certain foods such as coffee, wheat, rice, grapes and wine, some analysis procedures OTA strongly in force have been perfected on Coffee (Bandeira et al., 2008) grain cereal (Tournas et al., 2001), grapes and wine (EU, 2010). These studies represent a preliminary investigation to assess the factors affecting the coffee beans during the post-harvest treatment, to collect and identify post-harvest molds isolated from coffee and detect OTA producing strains.

MATERIALS AND METHODS

Sampling of coffee beans collection

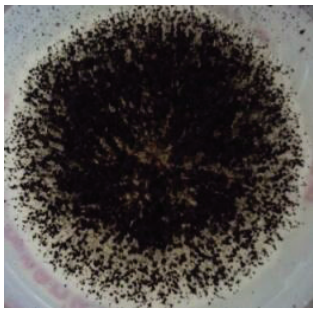
Surveys and sampling were undertaken during (the coffee year) coffee campaign 2008 and 2009. Sixteen varied samples are collected during survey in 2008: dried coffee cherry ground, green coffee beans in storage, coffee husks. Fifteen dried cherries were collected in 2009. The cherries present in all samples were hand-picked and have been processed by dried natural process, on different drying support: plastic film, ground, and cement. They were sampled in sterile plastic bags and stored at room temperature (30°C), then were brought to the research center.

Isolation and purification of fungi

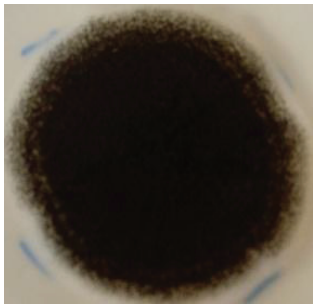
Fungi analysis were carried out on coffee sample of 300 g each one. Each sample was sub-sampled by taking three coffee cherries per 300 g of sample. Isolation was performed by direct plating technique (Perrone et al., 2007), on PDA medium (potatoes extract 4 g/L, dextrose 20 g/L, agar 15 g/L, distilled water 1 L). This technique allows growing most of the fungi present on the coffee cherry. From each sample, three coffee cherries per 300 g of sample were picked at randomly and applied to the surface of an agar medium PDA. Coffee cherries were incubated at 25 and 45°C for at least three days, after which we could see on the cherries and beans surface many fungi colonies of various genus (Figure 1a, b). Once purified on PDA agar, representative strains of each colonies were stored on PDA agar slants at 4°C. Determination of fungi genus was made by studying the morphological analysis.

Morphological analysis for identification

The phenotypic analysis of the filamentous fungi need references strains from international collection provided and by the Museum of the natural history of France. Morphological analysis of different fungi genus was applied by macroscopic and microscopic description of vegetative form called “the thallus” which allowed to differentiate the mycelium and conidia. Macroscopic studies are undertaken by observing fungi colonies with the naked eye and binocular microscope, to distinguish the main features of the fungal thallus. The various criteria studied in the mycelium are the color, the texture, the setbacks of the thallus and the contour. The criteria



(a)



(b)

Figure 2. Thalle of *Aspergillus* section *Nigri* on PDA**,** 25°C (5days (a) *Aspergillus carbonarius* group, (b) Aspergillus *niger* group.

considered for the spores are: powdery or granular, the color, the size, the density and the volatility. More apical speed of growth rate was measured to characterize the various fungal groups.

Microscopic study focused on the characteristics of the tubular filament of mycelium (septate or no septate), the shape of the conidial head (*Aspergillus*), the structure of the conidiophores (*Penicillium*), the presence of phialides and metulae at conidiogenous cells, and the size of conidia, their shape and external ornamentation (Botton et al., 1990; Samson et al., 2007). This stage is developed by, observing a piece of mycelium and conidial head, the objective (x40), between slide and cover slip in Bleu Cotton.

Determination of OTA-producing fungi by the method plug agar

OTA production and extraction from Aspergillus section Nigri isolates

Aspergillus section *Nigri* was investigated for OTA production. Strains were grown on CYA (Czapeck Yeast Agar) for 7 days at 25°C. Then OTA was extracted with methanol (Bragulat et al., 2001) following the methodology described by Sanchez-Hervas et al. (2008): three agar plugs of 5 mm diameter were removed of the central, the middle and the extremity of each colony, in triplicate. The agar plugs were ground in 900 µl of methanol. After 24 h of storage in the dark at 4°C, the extract was centrifuged at 1300 rpm (15 min) and filtered using a cellulose acetate filter brand Whatman (0.45 µm). The filtrate was stored at -20°C until HPLC analysis (Sanchez-Hervas et al*.,* 2008).

OTA detection and quantification from Aspergillus section Nigri extract

OTA quantification was carried out with an HPLC system by spectrofluorimetry (Nakadjima et al., 1997) using a fluorescence detector (Agilent Technologies 1200 serial). The detection was performed at the following wavelengths, 335 nm for excitation (λ exc) and 460 nm for emission (λ em). The wavelengths are specific for the fluorescent molecule. The fluorescence intensity depends on the concentration of the molecule. The detector is provided with a guard column (Nomura chemical) C18 column (Atlantis, 5 µm, 4, 6 x 250 nm). The following solvents were used for OTA analysis: acetonitrile, methanol and water (Sigma Aldrich), and the acetic glacial Carlo Erba. OTA standard was provided from a commercial stock solution of Ochratoxin A, 100 ng/ml (*Petromyces albertensis* assay ≥98%, Sigma Aldrich). The mobile phase (water/acetonitrile/acetic acid, 57/41/2) was pumped at an isocratic flow of 1 ml/min. OTA was identified by its retention time at 9.7 min according to a standard. The repeatability of the analysis was checked by standard solutions of OTA concentration and quantification by comparison with a calibration curve. The detection limit was 0,025 ng/ml.

RESULTS

Fungi genus classification

Genus identified in this collection (*Aspergillus* section *Nigri*, the *Aspergillus* section *Fumigati*, *Penicillium*, *Fusarium* and *Mucor*) are mesophilic because they have been isolated only at 25°C, except *Aspergillus* section *Fumigati* which have grown both 25 and 45°C. Macroscopic analysis of fungi aims to study the characteristics of the fungal thallus, such as the appearance of colonies, their relief and color. Microscopic examination focuses on structure of the mycelium (Cahagnier and Richard-Molard, 1998), the conidiophore (De Hoog and Guarro, 1995), conidiogen cells (De Hoog and Guarro, 1995) and conidiospores (Botton et al., 1990). The majority of *Aspergillus* section *Nigri* colonies on PDA at 25°C have a woolly, yellow or white mycelium, with a cream reverse color characterized by roughly tightened striations. *Aspergillus s*ection *Nigri* mycelium under optical microscope is septate. Following the color of spores and their dispersion on the thallus, *Nigri* section was divided in two groups. The thallus of the first group is white with long trunk and large spores scattered through the thallus. Conidial head is globular, biseriate and spores are large size and wall-warted (Figure 2a, b). This first group might similar to *Aspergillus carbonarius.* The second group consists of other strains of *Aspergillus* section *Nigri* of which criteria are different from *A. carbonarius* characteristics, whose spores are dark brown to brown, tightened against the other, lining the thallus and giving it a dense and powdery appearance.Their stipe is invisible to the naked eye (Figure 3a, b). The second group is represented by the sub-group of the complex *Aspergillus niger* aggregate

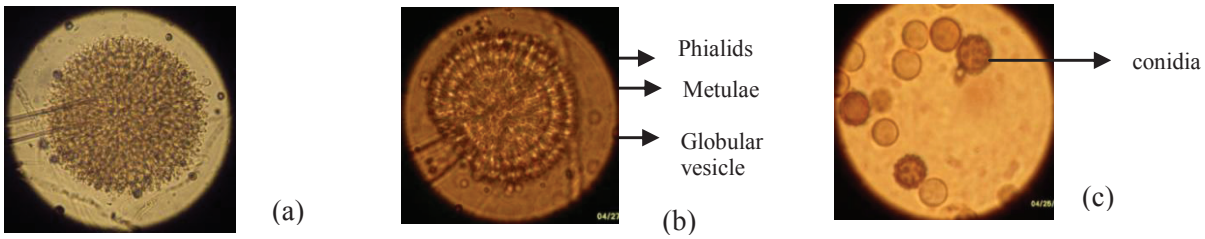


Figure 3. Microscopic structure of *Aspergillus* section *Nigri,* (x400): (a) Conidial head, (b) Biseriate conidial head, (c) Verruqueuse conidia.

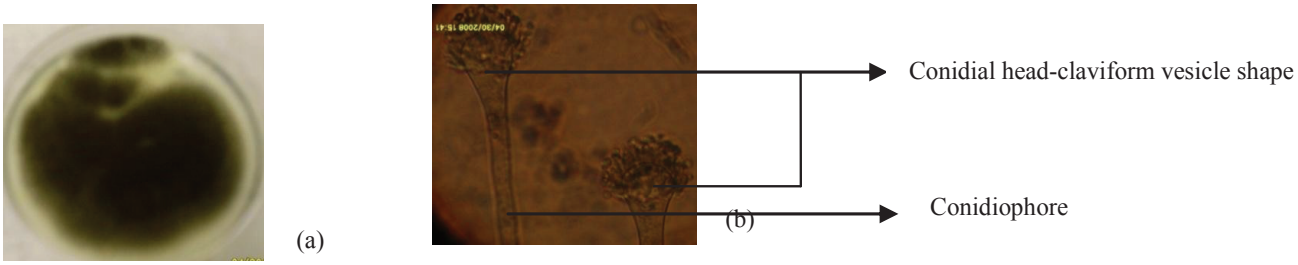


Figure 4. *Aspergillus fumigatus*: (a) Dark-green colony on PDA, 25°C and 45°C (5 days), (b) conidial head-claviform vesicle shape, Conidiophore (x400).

representing by *A. niger, Aspergillus tubingensis, Aspergillus foetidus, Aspergillus piperis, Aspergillus brasiliensis, Aspergillus ibericus, Aspergillus costaricensis, Aspergillus uvarum, Aspergillus vadensis* and *Aspergillus lacticoffeatus* (Samson et al., 2004).The conidia of the first group are wide spores warty-wall (Figure 3c) while in the second group they are mainly medium-sized and wall-echinulate (Albarca et al., 2004; Samson et al., 2007). Within the genus *Aspergillus* the comparison of the conidial head allowed to distinguish *Aspergillus* section *Nigri* which is globular type from *Aspergillus* section *Fumigati* which is clavatus type (Figure 4a, b). Following the mode of implementation of conidiogenous cells on the conidial heads, strains of *Aspergillus* section *Nigri*, were divided into two subgroups: the uniseriate and biseriate. Uniseriate strains count only one level of conidiogenous cells called phialides. On the other hand in biseriate strains, there are two levels of conidiogenous cells: metulae directly attached to the conidial head, carrying to their end phialides which are fruiting structures of conidia.

Colonies of *Aspergillus* section *Fumigati* have a fluffy appearance with a white mycelium, upholstered with green-dark spores (Figure 4a). The reverse is whitish and streaked. All strains of *Aspergillus* section *Fumigati* are uniseriate. Conidiogenous cells composed only of phialids cover a third party of conidial head which is claviform vesicle shape (Figure 4b). The mycelium is septate. They have the particularity to grow both at 25°C than at 45°C, making it mesophilic and thermophilic strains.

Fungi of the genus *Penicillium* have fluffy thallus covered by a powdery blue-green conidia (Figure 5a). The egg-shaped small conidia are hanging on a biverticille brush conidiophore with phialide hanging conidia (Figure 5b). *Fusarium* colony are pink fluffy. Microscopic structure shows egg-shaped conidia.The genus *Rhizopus* remarkably faster in growing whith an apical speed of growth rate which varies between 1.5 cm/day and 2 cm/day can get a diameter of 9 cm in 48 h and invade the Petri dish. *Rhizopus* colonies are very invasive on the surface of agar media (Figure 6a). Their thallus is white to grey and air-stringy with round-smooth-black structures called *sporangium*. Under optical microscope spores or sporangiospores are enclosed in the *sporangium* (Figure 6b).

Identification considered criteria (characteristics of the thallus, hyphae, conidiophores, conidiogenous cells, conidia) in this study are consistent with those defined by Botton et al. (1990) and Cahagnier and Richard-Molard (1998) and Hoog and Guarro (1995). Current taxonomic identification of filamentous fungi is based on micro-and macro morphological characteristics. The main identification markers in filamentous fungi are the cultural characteristics including colony on specific characteristics culture media (color, size, and shape), and development of sexual and asexual spore-forming structures, and / or physiological characteristics such as the ability to utilize various compounds as nitrogen and carbon sources (Glass and Donaldson, 1995; Cahagnier and Molard-Richard, 1998).

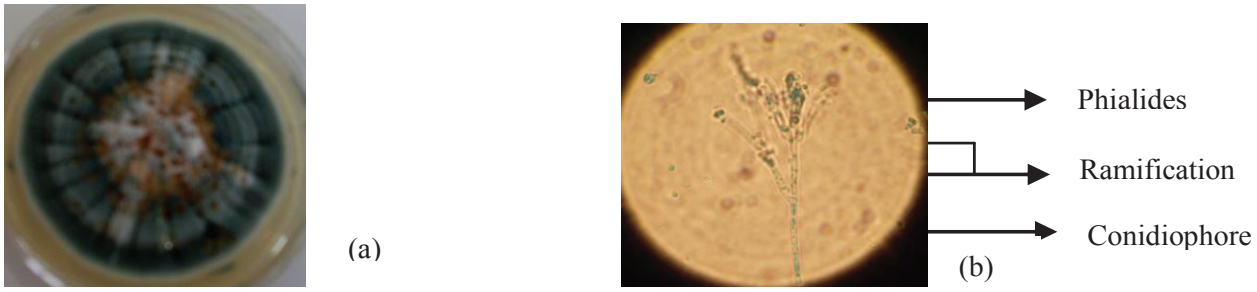


Figure 5. The genus *Penicillium*: (a) Colony on PDA, 25°C (5 days), (b) Biverticille brush structure (x400).

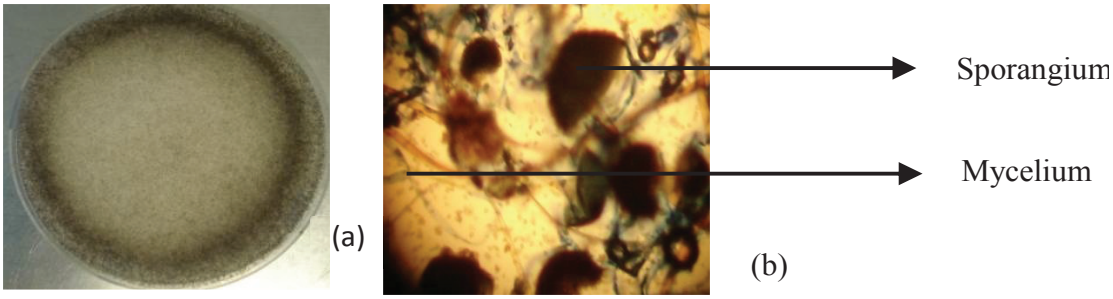


Figure 6. The genus *Rhizopus*: (a) Invasive colony of *Rhizopus*, 25°C, 48 h , (b) Microscopic structure of *Rhizopus* (x400).

The main genera isolated and identified in this study are *Penicillium*, *Aspergillus* and *Rhizopus*. The genus *Penicillium* is characterized by an apical speed growth rate very slow ranging from 0.3 cm/day and 0.5 cm/day, which makes them difficult to transplant during purification. Whereas *Aspergillus* grow faster with an apical speed of growth rate ranging from 1.25 to 1.9 cm/day. Optical microscope *Penicillium* differ to *Aspergillus* by their brush structure and conidia of round or ovoid arranged chain shape. As for *Aspergillus* they are characterized by *Aspergillus* conidial head whose head struck form or claviform is uniseriate or biseriate (Botton et al., 1990; Clenny, 2005). Macroscopic study by comparison with reference strains was able to differentiate the strains related to *Aspergillus carbonarius* strains belonging to *Aspergillus Niger* aggregate group by the size of the spores. All strains isolated during this study and classified the species *Aspergillus fumigatus*, have the particularity to grow between 45 and 50°C, growth temperature at which *A. fumigatus* is different from other related species (*Aspergillus fumigatiaffinis*, *Aspergillus novofumigatus*, *Aspergillus lentuli*) capable of growth at 10°C.

Occurrence of fungi on coffee

Results of mycological study on coffee samples was estimated two hundred and eighteen strains isolated mold spreading across the two years of campaigning in the following proportions 53% in 2008 and 47% in 2009 (Table 1). Several types of mold at different rates have been identified, there are: *Aspergillus* section *Nigri* (53%), *Aspergillus* section *Fumigati* (13%), *Penicillium* (10%), *Rhizomucor* (16%), *Fusarium* (4%), Other (4%). This diversity is strongly represented in 2008 by five genera (*Aspergillus* section *Nigri*, *Aspergillus* section *Fumigati, Penicillium*, *Rhizopus, Fusarium*) and only three (*Aspergillus* section *Nigri, Penicillium, Rhizopus*) in 2009, one year to the next the flora largest collection of mold remains the *Aspergillus* section *Nigri* to 28% for 2008 and 82% to the account of 2009. The same genus were isolated from coffee samples from different origins by Pardo et al. (2004) with a predominance of *Aspergillus* section *Nigri* at a rate of 65.4%. Genus *Aspergillus* was the major contaminant samples of coffee every year that to say 33% in 2008 and 84% in 2009.

State of fungi contamination

Table 1 shows the state of fungal contamination over the entire sample population on coffee sample of 300 g each one sampled in 2008 and 2009. This population are very heterogeneous features more by the diversity of the types of samples, the sampling site (in 2008) and the variation

Table 1. Estimation of fungal contamination on coffee with respect to the sampling population.

Sampling population/Drying/Region	Year	Average fungal contamination by sample group x 300 g	Anual average fungal contamination x 300 g	Statistical accuracy (error) (%)	Annual percentage of fungal contamination (%)	Annual percantage of *Aspergillus* section *Nigri* (%)	Percentage of *Aspergillus* section *Nigri* OTA producers in *Nigri* section (%)
Green Coffee Bean / West	2008	3.7					
Green Coffee Bean / Est	2008	1.8					
Dried cherries/West	2008	10	6.8 ± 1.47	1.34	53	28.5	18
Dried cherries/East	2008	7.7					
Dried shell peeled /West	2008	11					
Dried cherries/Cement/West	2009	7.3					
Dried cherries/Plastic/West	2009	5.7	7.25 ± 1.58	1.7	47	82	19
Dried cherries/Plastic film/West	2009	5.7					
Dried cherries/Ground /West	2009	5.7					
Overall balance sheet 2008/2009			7.03 ± 0.726	3			

of the drying yard (in 2009), than by their origin. Groups of samples are classified according to the type of samples (dried cherries, nuts, green beans) and types of post-harvest treatments or sampling sites (plantations, warehouse, shellers, drying area treatments, no-determined). The sampling was conducted according to a completely randomized to a repeat. One treatment (isolation of mold at 25°C on PDA agar by direct contact) has been carried out on one coffee sample batch of 300 g for qualitative post-harvest filamentous fungi research. Significance or accuracy of fungal contamination in relation to the sampling population considered was obtained by the inverse of the variance. The entire sample population is heterogeneous; there is loss of precision on the actual level of fungal contamination. In 2008, there are 98.6% chance that the average fungal contamination varies in a range from 6 to 9 fungi per batch of 300 g of coffee samples. Whereas in 2009 the level of fungal contamination estimated at between 5 to 8 fungi per batch of 300 g coffee samples is 98.3% reliable. The overall fungal contamination evaluated on campaigns in 2008 and 2009 is 97% reliable and is estimated at 7.03 of fungi in one coffee sample batch of 300 g.

There is no significant difference between the mean of fungal contamination in 2008 and 2009 as the mean difference (0.45) was less than the least significant difference (119.16). If the level of fungal contamination does not vary from one year to another it means that the factors of change that have occurred from one year to the other during the sampling season, sampling sites, the climate and the drying conditions (rain, prolonged sunshine...) did not influence fungal contamination. However, some groups of samples like dried cherries/west in 2008 and cement dried/cherries/west in 2009 are representative of the level of fungal contamination evaluated during these years. There is no correlation between the type of samples, post-harvest treatments and the level of fungal contamination. If the level of fungal contamination does not change, that means that the variation factors are environmental factors such as climate, varing little from east to west from one year to another.

The results obtained from this evaluation can not allow to establish a correlation between the level of fungal contamination and different drying racks. Contrary to the recommendations of good agricultural practices all areas of drying without distinction have the same level of fungal

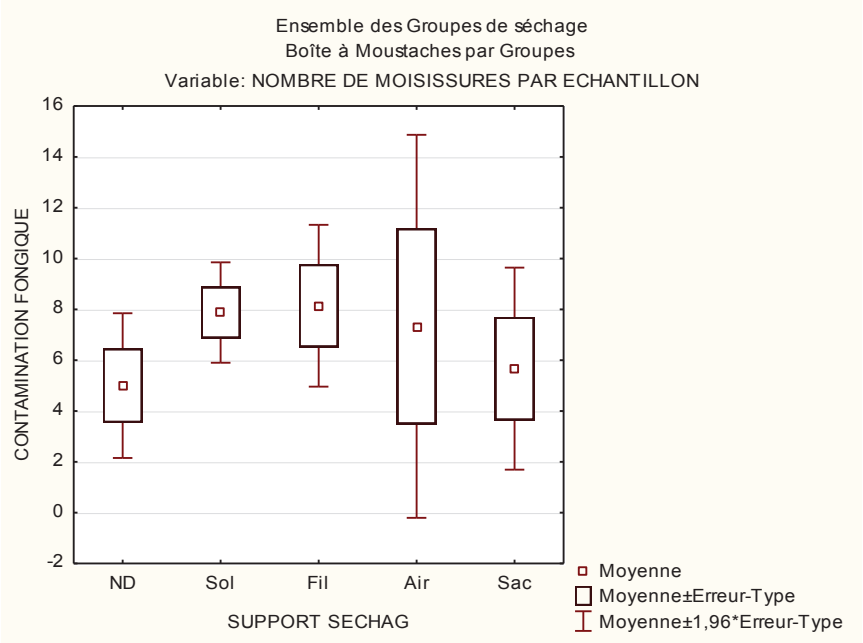


Figure 7. Fungal contamination rate according drying yard.

contamination. This analysis is supported by (Figure 7) for studying trends by showing the variability of fungal contamination on different drying area: non-drying determined (ND), the drying ground (Sol), the drying cemented area (Air), drying on a plastic film (Fil). On non-determined supports the average rate of fungal contamination is 5 fungi per sample. It can vary within a confidence interval of 3.8 to 6.5 molds. The extent or limit of dispersion (if the parameters vary) varies between 2.1 and 7.8. It means that the average of fungal contamination in case of Good Agricultural Practices or implemented HACCP quality methods can fall to 2 fungi per samples of 300 g. In extreme cases of stress or slackening during good agricultural practices application (use of improved technology such as rack, the plastic film and maintenance, protection against moisture coffee, aerated storage drying ...) fungal rate contamination may progress to 7.8 per sample of 300 g. It is observed that there is an inconsistency between the means of contamination and post-harvest treatments during drying. Indeed considering the customs of the farmers in some villages healthy of drying areas is no longer a distinctive drying quality because other drying areas (the cemented area, the bag and the film plastic) trampled by farmers are subject to soil contaminants. The highest risk of exposure to contamination was characterized by fruit contact with the soil, constituted by the fraction coffee swept from ground, and by inadequate post-harvest handling of the product during drying in ground patios. Ground patios must be avoided, since soil is the natural habitat of ochratoxigenic fungi and other microorganisms as well-other (Batista et al., 2009).

OTA profil of black *Aspergillus* section *Nigri*

Mycotoxin analysis revealed that some strains of *Aspergillus* section *Nigri* isolated on robusta coffee in Côte d'Ivoire are able to produce OTA (Table 2). OTA production is brought out by a pic at a retention time of 9,639 (Figure 8). From all strains evaluated during 2008 and 2009 seasons, 11% of OTA producers were detected with levels ranging from 0.3 to 56 µg/g of czapeck yeast extract agar medium. The isolates considered as OTA producers presented a retention time similar to that of OTA standard. It is common to find in *Aspergillus* section *Nigri* producing Ochratoxin A.The results of the most sophisticated chromatographic procedures depend on the efficiency of the prior sample preparation, in particular on sampling, extraction and the further treatment of the extract, including any purification. As a large number of interfering compounds present in samples may contaminate the primary sample extract, these components must be removed as completely as possible for most method applications (Krska, 1998). The study of the production potential of OTA shows that strains do not possess the same level of concentration of Ochratoxin A. Some strains of the same sample, levels of OTA concentrations are sometimes close to each other (sample 30) or very distant (sample 29).

DISCUSSION

In coffee contamination by mold *Aspergillus* including *A. carbonarius* and *A. niger*, has been reported in several

Table 2. *Aspergillus* section *Nigri* OTA producers.

Samples number	Sample type	OTA producers/number of *Aspergillus* section *Nigri*	OTA Concentrations (µg /g of agar medium)	Year
	Coffee cherries	1/3	8.27	2008
Ech13	Coffee cherries	1/3	6.56	2008
	Coffee cherries	1/3	10.3	2008
	Coffee cherries	1/3	38.96	2008
Ech14	Coffee cherries	1/3	45.41	2008
	Coffee cherries	1/3	56.4	2008
Ech18	Coffee cherries	1/3	32.46	2009
	Coffee cherries	1/3	18.58	2009
Ech20	Coffee cherries	1/9	35.4	2009
	Coffee cherries	1/9	9.1	2009
Ech24	Coffee cherries	1/5	8.03	2009
Ech27	Coffee cherries	1/6	0.31	2009
Ech28	Coffee cherries	1/8	1.91	2009
	Coffee cherries	1/5	4.2	2009
Ech29	Coffee cherries	1/5	40.5	2009
	Coffee cherries	1/5	27.6	2009
	Coffee cherries	1/7	9.3	2009
Ech30	Coffee cherries	1/7	9.36	2009
	Coffee cherries	1/7	10.98	2009
	Coffee cherries	1/9	20.81	2009
Ech31	Coffee cherries	1/9	11.27	2009
	Coffee cherries	1/9	1.3	2009

countries (Perrone et al., 2007). *Aspergillus* are responsible for many diseases in plants and agricultural products from harvest to process transformation via by post-harvest treatments (Perrone et al., 2007). In studies conducted in Brazil it is mainly *Aspergillus* section *Nigri* that are contaminants of coffee and individuals *Aspergillus niger* (83.3%) (Martins et al., 2003) and *A. carbonarius* (Magnani et al., 2005). Similarly Pardo et al. (2004) have isolated a high percentage of *Aspergillus* section *Nigri* (65.4%) on coffee samples. In Vietnam, Leong et al. (2007) were isolated from coffee cherries robusta and arabica, strains of *A. carbonarius*. Results with the *Aspergillus* section *Nigri* were published as dominant for samples of arabica coffee beans collected in Brazil (Batista et al., 2009). According to Albarca et al. (2004), many species within *Aspergillus* section *Nigri* are OTA producers, mainly *A. niger* and *A. carbonarius*. Reported results (Table 2) on OTA production vary between 0.3 to 56 µg/g. It similar to others as Riba et al. (2008) (0.01 to 0.17 µg/g) and Sanchez-Hervas et al. (2008) (0.2 to 90 µg/g), who used the same method (Filtenborg and Frisvad, 1980; Batista et al., 2003) to extract OTA from filamentous fungi isolated on coffee and cocoa beans. Ochratoxin A is one of the most dangerous mycotoxins produced by certain important filamentous fungi such as *A. ochraceus* (Van Der Merve et al., 1965), *A. carbonarius* (Teren et al., 1996), with some isolates of *A. niger* (Abarca et al., 2001) and *Penicillium verrucosum* (Schmidt-Heydt et al., 2010). In case of multianalyzes screening an immunochemical biosensor assay for the detection of multiple mycotoxins (Aflatoxin B1, Zearalenone, Ochratoxin, DON and Fumonisins) has been developed (Van der Gaag et al., 2003). The detection limits of the multiple assays are 0.2 ng/g for Aflatoxin B1, 0.01 ng/g for Zearalenone, 0.1 ng/g for Ochratoxin A, 50 ng/g for Fumonisin B1 and 0.5 ng/g for DON. For the sample extracts where OTA could be detected, it is possible to increase the sensitivity of the assay using alternative techniques including the detection threshold varing within a range of values below the limit

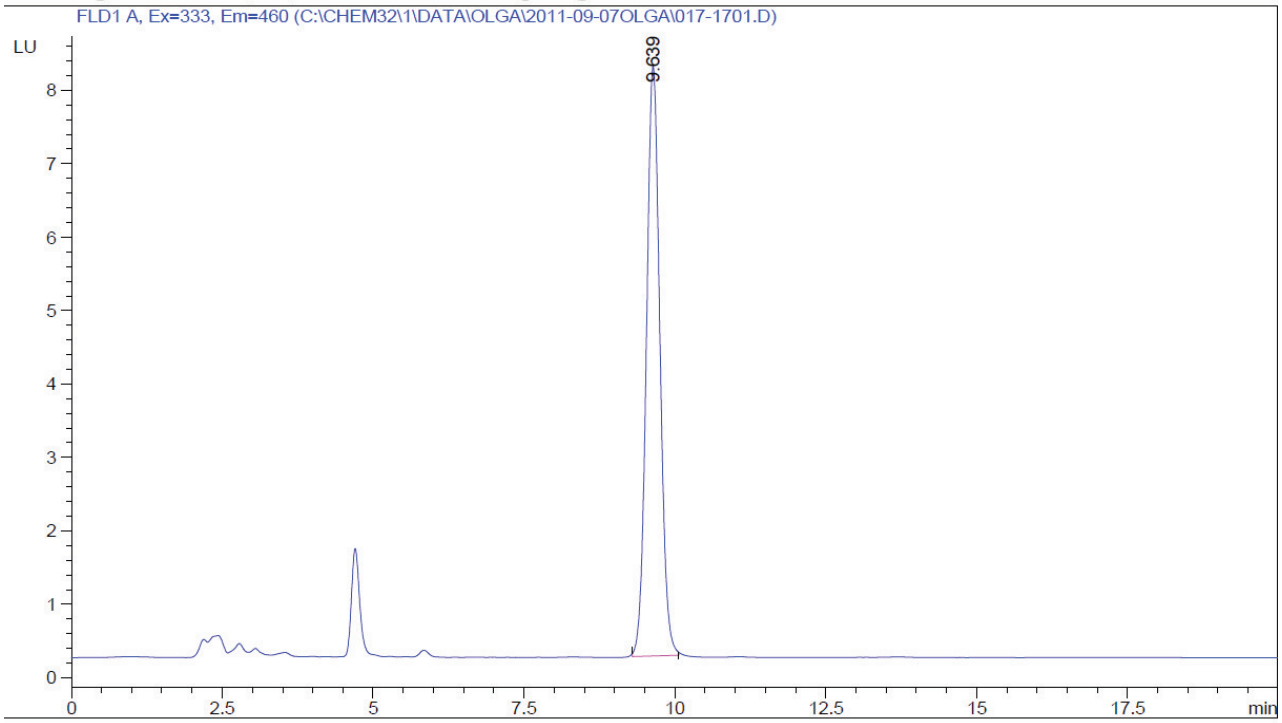


Figure 8. Ochratoxin A peak of strain DO 127 extract.

of HPLC detection. These emerging technologies such as biosensors are based on principles of receiving wavelength signals emitted by the molecule on optical surfaces like (e.g. surface plasmon resonance, SPR, and evanescent wave fibre optic) et acoustic (e.g. quartz crystal microbalance).This type of approach has been reported for the determination of Ochratoxin A in liquid food products (Hauck et al., 1998). The assay was based on competition of free Ochratoxin A (test sample) and a sensor surface immobilized conjugate of ochratoxin with the corresponding antibody, the resulting binding of excess antibody to the surface being detected at a resonance frequency of 20 MHz. The method has a linear range of 2 to 100 ng/ml (Patel, 2004). The analytical method based on capillary electrophoresis with fluorescence detection capillary zone electrophoresis coupled with laser-induced fluorescence CZE-LIF) can detect traces of mycotoxins whose resolution was induced by the fluorescent lazer or by exploiting the fluorescent properties of the test molecule and its derivatization with fluoroflore isothiocynate Fluorescein isothiocynate (FITC). This analytical method applied to fumonisins separation and quantification in corn samples involved the following step: after extraction of fumonisins from corn samples with methanol/water, they were isolated using an Immuno Affinity Column. Then the extract was traited by derivatization with FITC and analyzed further by CZE-LIF. The CZE-LIF method was comparable in sensitivity to that of an HPLC method (Maragos et al., 1996). Most methods utilize the fluorescent properties of OTA for subsequent detection and quantification. However, the complex nature of the matrices from which OTA is extracted gives rise to the potential for interference with the fluorescence signal and, therefore many laboratories have used a secondary technique such as liquid chromatography with mass spectrometric detection (LC-MS) to confirm the analytical results obtained from their primary method. Some methods have used mass spectrometry (MS) or immunoassay instead of fluorescence to quantify OTA. For the most part, the recoveries reported using the various methods are above 80 or 90% and detection limits are in the low parts per billion (Scott, 2002).

Fungal diversity observed in 2008 compared to 2009 means that fungi isolated on coffee samples in Ivory Coast would be subject to seasonal and may vary from year to year. But it consists essentially of mycoflora with a predominance of *Aspergillus* section *Nigri.* Fungi rate in coffee samples varies with samples types, geographical environment and drying support used. Fungal contamination was probably due to the reduction of water activity which inhibits the growth of other microbial groups e.g. bacteria and yeasts. Humidity and chemical composition of coffee beans, crop's environmental conditions and product management can influence development of microorganisms and their metabolic activity. The adoption of the Danger and Critical Control Points Analysis System and Good Agricultural Practices

(GAP) will significantly influence not only the reduction of fungal contamination risk under the conditions of coffee fruit and bean deterioration but also the reduction of OTA (Batista et al*.,* 2009). The difference in genus diversity can be explained by the difference of homogeneity in the samples. During the 2008 campaign sampling certainly is distinguished by the diversity of the types of samples (dried cherries, green beans and wet and dry shells), sampling sites (plantations, box of farmers, shellers, warehouse) but especially in the area of origin (West and East) coffee samples. This distinction is the more remarkable because the conditions of post-harvest treatment specifically drying and storage are unknown. Sampling during 2009 is more homogeneous than last year because all packages of cherries sampled from a single village in the West, had been taken during drying, whith farmers in the same neighborhood. The change in physico-chemical parameters (temperature, Aw, pH) subjected to weather randomness (alternating rain and sunshine) and the succession of the seasons occurring during post-harvest processing and endogenous flora in the area coffee growing, are sufficient factors to guide the growth of some fungal flora on coffee cherries. Proliferation of fungi in coffee is due to severe faults in harvesting and storage practices like variation of physico-chemical factors (temperature, water activity, humidity) during storage and transport of cherries or coffee beans. It concerns good agricultural practices particularly cherries drying-tickness on drying support (Kouadio et al., 2012), drying process (Velmourougane et al., 2011; Magan and Olsen, 2004) and storage conditions (Bucheli and Tanikawa, 2002). For example in Ivory Coast the effect of coffee cherries quantity put out for sun drying on the kinetics of the drying, fungal growth and Ochratoxin A production was evaluated. The results showed that the more coffee cherries quantity on the drying area was important, the slower they dried. Then the slowness of the drying led to the increasing of fungal development and Ochratoxin A production in the cherries (Kouadio et al., 2012). Their microscopic and macroscopic characteristics are consistent with the description given by (Rapper and Fennell, 1965; Rinyu et al., 1995; Hong et al., 2005; Samson et al., 2006). *Aspergillus section Nigri* are very widespread in the world. Although their primary source is the ground, they are among the major contaminants of foods and their raw material (wheat, coffee, cocoa) (Perrone et al., 2007). Several species such as *A. carbonarius, A. japonicus*, *A. aculeatus* and some variants of the Niger aggregate group are responsible for the post-harvest fruit (apples, peaches, grapes, figs, tomatoes, melons, ...), plant (onions, garlic, yam), and nuts (peanuts, pecans, pistachios, ...) alteration (JECFA, 2002).

Fungal contamination of coffee should not be closely related to mycotoxin production on coffee. To ensure certainty it would be necessary for the determination of OTA in coffee samples. Regarding coffee samples processed by dry method, the results in Table 2 showed that OTA fungi producers have been isolated only from dried coffee cherries. Then if deshusking and despulping are carried out, OTA and fungi contamination in coffee samples would be reduced. Indeed, Batista et al. (2009) concluded as a result of their work that fungi and OTA contamination are concentrated in the skin until processing. According to Bucheli and Tanikawa, (2000), coffee bean skin is the main substrate for the development of OTA fungi. This would explain the fact that potentially ochratoxinogen filamentous fungi were isolated only on dried cherries. Although Ochratoxin A is a mycotoxin of storage, the majority of strains tested in this work, are isolated from coffee cherries during drying in the farms. During storage, in high humidity condition accumulation of toxin is the possible, but the minimum moisture does not guarantee the absence of OTA production. However it takes less moisture for the fungus growth. Many research studies have shown that species of *Aspergillus* are natural contaminants of coffee and mycotoxins are present on coffee from farming to storage (Nakajima et al., 1997; Silva et al., 2000; FAO, 2006b). Indeed, fungi such as *A. ochraceus* and *Aspergillus* section *Nigri* spp. are responsible for the production of OTA in coffee during drying and storage (Belli et al., 2005). The fungal contamination of the coffee was analyzed by Paterson et al. (2004) in the context of variable climate and evolving. The main climatic factors affecting agriculture in this case the weather variability, seasonality, average rainfall, water availability, and the dynamics and distribution of pests in cases where they are not controlled, as well influence the post-harvest fungal contamination (Bourgeois, 2009). The amount of OTA was dependent on the latitude of the production region: the lower the latitude, the more frequent the occurrence and the greater the concentration. The considerable climatic differences, related to geographic regions, influenced fungi contamination and OTA production in a conclusive manner (Zimmer and Dick, 1996).

Like other foods (cereals, nuts, peanuts, cocoa, dried fruits, cheese, salted and cooked meal, pastry, spicies, ...) (Pfohl-Leszkowicz and Castegnaro 1999) coffee cherries and beans and coffee husk were exposed to fungal contamination during different phases of development, harvesting, preparation, transport and storage. Species were isolated from pure cultures. Phenotypic analysis of the investigated strains in comparison to reference strains do not provide sufficient information to distinguish between species, because of the great phenotypic variability filamentous fungi and especially those of the genera *Aspergillus* (Raper and Fennell, 1965; Al-Musallam 1980). Indeed molecular methods with DNA-based tools will are useful to examine phylogenetics and systematics of fungi (Bruns et al., 1991; Bowman et al., 1992; Glass and Donalson, 1995). Post-harvest treatment well performed reduces coffee

beans fungal contamination. Therefore if the storage takes place in good conditions (good ventilation, optimum moisture, and adequate temperature) green coffee beans were relatively healthy for export. So there were corrections to be made during post-harvest processing of coffee, including the application of good agricultural practice to reduce fungal contamination and mycotoxins production. Also improving the drying process by natural means, including an intermediate stage of pulping and degumming wet may prevent the growth of mold and therefore OTA production during the post-harvest treatment. This will prevent fungal contamination in coffee cherries skin. Because less coffee cherries are infected, the less will be the green coffee beans ready for export and healthy for consumers. Added to this is the search for bioprocess antifungal to fight against the growth of mold on the coffee samples during the post-harvest treatment. In other fungi studied in this chapter have been the subjects of particular study in search of antifungal lactic acid bacteria. The potential antifungal activity of *Lactobacillus plantarum* against germination and mycelial growth of certain species of *Aspergillus* section *Nigri* was confirmed. The results of this work are published in the journal Anaerobe (Djossou et al*.,* 2011). The ageing of coffee plantations has resulted in and the dropping of coffee plantations by the farmers class in favor of new crops export such as cashew tree, *Hevea brasiliensis, cotton, oil palm ...* . It is of great interest for economic actors in the coffee sector to propose new plant varieties more productive and profitable coffee trees.

Conflict of Interest

The authors have not declared any conflict of interest.

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Exports and market power of the soybean processing industry in Brazil between 1980 and 2010

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The objective of this research was to identify whether the majors of the soybean production chain in Brazil have conditions to design their market power and capture part of the economic surplus farmers? In this context, based on the analysis of price elasticity, drawn from the postulates of neoclassical microeconomic theory, it was found that exports of soybeans and soybean meal are inelastic to price. Therefore, companies are able to exercise their market power, mainly because they are protected by barriers to entry. In contrast, exports of soybean oil are price elastic and therefore, the soybean industry tends to seek profit on transactions with farmers to improve their competitiveness in the soybean oil segment. As a result, the Brazilian government should use mechanisms of economic policy to foster competition in the market.

Key words: Soybean supply chain, agribusiness, market structure, export function, price elasticity.

INTRODUCTION

Given the economic importance, social significance and contribution to the national and international food security, the soybean (*Glycine max* (L.) Merr.) production chain is one of the most important in Brazil's agribusiness. Explanation for this includes the development of new technologies resulting from public and private investments, which have revolutionized the management practices for soybean cultivation (Giordano, 1999; Brum, 2002; Rezende et al., 2003; Embrapa, 2004). On the other hand, this process of development resulted in the constitution of oligopolies and oligopsony (Lima, 2012; Costa and Santana, 2014), such as the segment of transgenic seeds (Costa and Santana, 2013), agricultural machinery, fertilizers and pesticides (Costa, 2012).

Thus, trade relationships between farmers and the agricultural inputs industries as well as the processing agroindustry began to take place in an imperfect market environment, which leaves farmers with no choice but to buy inputs from oligopolistic companies and market their production in an oligopsonized market structure (Wesz Junior, 2011; Sediyama et al., 2013).

While soybean culture may contribute to the development of many regions located in areas of livestock farming in Brazil. The possibility of losing competitiveness as a result of market failures should be

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evaluated. Thus, the present research aimed to examine the importance of variables such as exchange rate, income and price, because about 48% of soybeans, 52% of soybean meal and 23% of soybean oil of the country's production of the soy complex are for the export market (Abiove, 2011).

Given this, the key issue of the present research is to find out whether the companies that determine governance in the supply chain can use their dominant position to exercise their market power by capturing the economic surplus from soybean farmers. The analysis, based on instrumental neoclassical microeconomics begins with the econometric estimation of a function of exports to the markets of soybeans, soybean meal, and soybean oil.

Existence and exercise of market power

The neoclassical economics theory has legitimated the concept that the free enterprise of firms and consumers lead the economy to an efficient allocation of resources, mainly by the first theorem of the Welfare Economics: "any general competitive equilibrium, regardless of the initial allocation of resources, maximize the well being of society" (Pareto, 1988: 13). In this context, the hypotheses devised by Smith (1988) and Ricardo (1988) that the free enterprise market is capable of leading the economic system to an improved allocation of resources was confirmed by Pareto (1998).

However, the theoretical assumptions of economic rationality, information domain and free entry and exit of suppliers and buyers, which features efficient competition, cannot be seen in all market sectors. In this context, Miller (1981) demonstrates that the exercise of market power is related to the ability of the industry to set market prices for factors and products above the marginal cost. In general, this practice is directly related to conditions involving: 1) price elasticity of demand; 2) number of competitors; 3) degree of competition among firms.

In the same line, Possas (1996: 4) identified that "the exercise of market power via price implies a demand with elasticity sufficiently low so that a price rise (and reduction of quantity) will result in increased profits – without which the strategy of price increase would not make sense" (author's translation). Pindyck and Rubinfel (2007) also demonstrated that the smaller the number of firms competing in the market, the greater the oligopoly; the lower the competition between firms, the greater their ability to raise profits by price manipulation.

Possas (1996) and Pindyck and Rubinfeld (2007) found that the degree of product substitutability and income are also important elements, because the greater the possibility of substitution the higher the price elasticity and the lower the ability of setting prices. By analogy, the more inelastic the farmers' supply the greater their risk of exposure to the market power of agro-industrial corporations. Such issues are important because the exercise of market power leads to the loss of economic efficiency and, above all, to the transfer of part of the consumers' surplus (or producers' surplus) to a select group of leading companies (Ferguson and Ferguson, 1994; Possas, 1996, 2002; Pindyck and Rubinfeld, 2007). As a result, it has been consolidated in the field of economic and legal sciences; the notion that markets must be subject to the establishment of regulations (legal norms) and political instruments to assure competition.

While the likelihood of exerting the dominant power varies according to price elasticity and that a significant part of the Brazilian production of soybeans, soybean meal and soybean oil is destined to the international market, the analysis of the possibility of market power via prices was performed according to the econometric estimation of the trade flow.

The trade flow model studies was conducted by Goldstein and Khan (1978), Dornbusch and Fischer (1994), Castro and Cavalcanti (1997), Cavalcanti and Ribeiro (1998), Onunkwo and Epperson (1999), Zini Júnior, (1995), Barros et al. (2002) and Santana (2002), who defined that the trade balance (difference between exports and imports) is a variable that is explained mainly by the real exchange rate, domestic income and the rest of the world's income.

In this sense, "the trade balance depends positively on the real exchange rate and on the income of the rest of the world and negatively on the domestic income" (author's translation) (Zini Júnior, 1995: 138); the real exchange rate tends to interfere directly with the trade balance because it causes relative changes in currency expenditures for the purchase of goods (Krugman and Obstfeld, 2010: 307); the domestic income should make up the model because it is directly related to domestic consumers' expenditures so that rises in this variable tend to result in increased outlays, including imports; and the increased international income tends to impact domestic exports. On the other hand, the price variable is directly related to changes in the consumers' purchasing power (microeconomic concept of income effect) and consumption decisions (microeconomic concept of substitution effect), widely studied and described in the work by Varian (2006) and in the main microeconomics textbooks.

MATERIALS AND METHODS

The Multiple Linear Regression (MLR) was used to estimate the econometric equations. As a technique, "the regression analysis is the most important econometric method" (author's translation) (Santana, 2003), because it allows to identify the effects that some variables exert on others.

In this case, in which data are from historical series, the Augmented Dickey-Fuller (ADF) test was used to identify the degree of the series integration and stationarity. According to Gujarati (2006), the ADF test consists of estimating a regression such as:

$$\Delta Y_t = \beta_1 + \beta_2 t + \delta Y_{t-1} + \sum_{i=1}^{m} \alpha_i \Delta Y_{t-i} + \varepsilon_t \quad (1)$$

Where: ε_t is a pure blank series, and $\Delta Y_{t-2} = (Y_{t-2} - Y_{t-3})$, etc., α and β are the parameters.

Thus, first the stationarity hypothesis was tested in level, without intercept and trend component. Subsequently, the stationarity hypothesis was assessed with intercept and trend component. Tests for serial autocorrelation and heteroscedasticity were not performed because the equations were calculated by the Generalized Method of Moments (GMM), considered robust to correct automatically these problems, if any (Hansen, 1982). The importance of the tools in the GMM estimation was assessed by the J-statistics introduced by Hansen. From time series the econometric models were adjusted for analysis of the markets of soybeans (Equation 2), soybean meal (Equation 3) and soybean oil (Equation 4):

$$QTXG_t = \alpha_0 + \beta_1 PXG_t + \beta_2 (PXG_t)^2 + \beta_3 PXF_{t-1} + \beta_4 PXO_t + \beta_5 REXCHANGE_t + \beta_6 GDPBR_t + \beta_7 PIBAS_t + \varepsilon_{gt} \quad (2)$$

$$QTXF_t = \alpha_0 + \beta_1 PXF_t + \beta_2 (PXF_t)^2 + \beta_3 PXG_t + \beta_4 PXO_t + \beta_5 (REXCHANGE_t)^2 + \beta_6 GDPBR_t + \beta_7 GDPAS_t + \varepsilon_{ft} \quad (3)$$

$$QTXO_t = \alpha_0 + \beta_1 PWSOYOIL_t + \beta_2 REXCHANGE_t + \beta_3 GDPBR_t + \beta_4 GDPEU_t + \beta_5 PWPALMOIL_t + \beta_6 PWCANOLAOIL_t + \beta_7 PWSUNFLOWEROIL_t + \varepsilon_{ot} \quad (4)$$

Where:

Endogenous variables

$QTXG_t$: Total exports (10^3 kg) of soybeans from 1980 to 2010.
$QTXF_t$: Total exports (10^3 kg) of soybean meal from 1980 to 2010.
$QTXO_t$: Total exports (10^3 kg) of soybean oil from1980 to 2010.

Exogenous and instrumental variables

PXG_t: Average export price (USD/metric ton) of soybeans (USD of 2010; FOB, Brazil).
$PXG_t{}^2$: squared PXG_t.
PXF_t: Average export price (USD/ metric ton) of soybean meal (USD of 2010, FOB, Brazil).
PXF_{t-1}: PXF_t lagged over one period
$PXF_t{}^2$: Squared PXF_t;
PXO_t: Average export price (USD/ metric ton) of soybean oil (USD of 2010, FOB, Brazil).
$REXCHANGE_t$: Real exchange rate: $REXCHANGE_t = NEXCHANGE_t(P_t^*/P_t)$, where $NEXCHANGE$ is the BRL/USD nominal exchange rate, P_t^* is the consumer price index in the USA and P_t is the general price index – domestic supply, Brazil;
$GDPBR_t$: Real Gross Domestic Product *per capita* in Brazil in year *t*, calculated according to the Purchasing Power Parity;
$GDPAS_t$: Real Gross Domestic Product *per capita* in ASEAN5 in year *t*, calculated according to the Purchasing Power Parity and used to represent the income of the Asian continent as a whole;
$GDPEU_t$: Real Gross Domestic Product *per capita* in the European Union in year *t,* calculated according to the Purchasing Power Parity.
$PWSOYOIL_t$: Average price of world exports of soybean oil in USD/metric ton in 2010, obtained by the quotient between the exported value in USD/ metric ton and the amount exported in 10^3 kg by all countries.
$PWPALMOIL_t$: Average price of world exports of palm oil in USD/metric ton in 2010, obtained by the quotient between the exported value in USD and the amount exported in metric ton by all countries.
$PWCANOLAOIL_t$: Average price of world exports of canola oil in USD/metric ton in 2010, obtained by the quotient between the exported value in USD and the amount exported in metric ton by all countries.
$PWSUNFLOWEROIL_t$: Average price of world exports of sunflower oil in USD/metric ton in 2010, obtained by the quotient between the exported value in USD and the amount exported in 10^3 kg by all countries.

Parameters

α_i is the general intercept value of the equation; β_j are the parameters to be estimated.

Error term

ε_{it} is the random error term of the equation *i* (soybeans, meal and oil).

It is expected that the parameters *β* relating to the price variables of the products present negative sign as a function of income. Similarly, in cross relationships between the exported amount of goods *x* and the price of goods *y*, it is expected a positive sign for parameter *β*, indicating, for example, that a rise in the export price of soybeans tends to stimulate Brazilian exports of soybean meal, *ceteris paribus*. The parameter sign for the real exchange rate variable must be positive, indicating that exports tend to increase to the extent that the exchange rate suffers depreciations, *ceteris paribus*, as emphasized by Dornbusch and Fischer (1994), Zini Júnior (1995) and Santana (2002). Finally, the principles of the trade flow model point to inverse relationships between exports and domestic income and constant relationships between exports and external income.

For a better detailing of the market of edible oils and analysis of the existence of long-term integration between the diverse types of oil supply, the Johansen's co-integration test was performed, methodologically detailed by Johansen (1988). According to Santana (2003: 432), "the co-integration equation can be interpreted as the long-term relationships between variables" (author's translation). Thus, the set of time series $PWSOYOIL$, $PWPALMOIL$, $PWCANOLAOIL$, $PWSUNFLOWEROIL$ was submitted to the co-integration analysis to confirm the existence of long-term linear combination.

RESULTS

The markets for soybeans, soybean meal and oil were

Table 1. Regression to explain export of soybeans from Brazil.

Variable	Coefficient	Std. Error	t-Statistic	Prob.
C	-27.961.859.00	5.640.624.00	-4.9572	0.0001
PXG	-36.113.72	17.888.78	-2.0188	0.0559
PXG²	40.87	19.47	2.0997	0.0474
PXF_{-1}	22.055.86	5.205.36	4.2371	0.0003
PXO	-2.350.87	856.25	-2.7455	0.0118
REXCHANGE	2.902.130.00	432.684.90	6.7072	0
GDPBR	2.527.05	1.284.22	1.9677	0.0618
GDPAS	5.206.33	2.123.76	2.4514	0.0226
R-squared	0.9754	Mean dependent variable		10.280.953.00
Adjusted R-squared	0.9676	S.D. dependent variable		9.430.533.00
S.E. of regression	1.696.636	Sum squared residual		6.33E+13
Durbin-Watson statistic	1.4432	J-statistic		1.51
Instrument rank	9	Prob(J-statistic)		0.22

examined according to independent, single-equation econometric models.

Exports and market power in the soybeans market

The results of the Augmented Dickey-Fuller (ADF) test show that all time series are integrated of order one I (1). The analysis of the exports and the likelihood of an existing market power were made by the estimation of Equation (2). The instrumental matrix that aggregated the direct effects of PXG, PXG2, PXFt-1, PXO, REXCHANGE, GDPBR and GDPAS and the indirect effects of PXF2 calculated both the standard deviation and covariance.

One of the GMM characteristics is the choice of coefficients in such a way that the residues are orthogonal to the instruments used. In the case under study, the P-value (J Statistics) of 0.219 confirms the orthogonality of the tools.

The probability value and t-statistics confirm that all estimated parameters are statistically different from zero: GDPBR at 10%, PXG and GDPAS at 5% and PXF and REXCHANGE at 1%, as can be seen in Table 1.

The adjusted R² value of the regression indicates that 96.76% of the changes in the exported amount of soybeans are explained by the set of exogenous and instrumental variables, that is, by the variables that represent the export price for soybeans, the real exchange rate and the domestic and international income.

The coefficient of price elasticity of soybean exports at the level of 0.1955 (Equation 5) indicates that the price variations of soybeans have little influence on the amount exported of the same product.

$$E_{P_g} = \frac{dQ}{dP} * \frac{\overline{Px}}{\overline{Qx}} = (b + 2c\overline{Px}) * \frac{\overline{Px}}{\overline{Qx}} = -36.113{,}72 + 2 * 40{,}87 * 374{,}81 * \frac{374{,}81}{10.280.953} = -0{,}1955 \quad (5)$$

In this context, for every 10% rise in the export price of soybeans, a decrease of 1.96% is expected in the amount exported, *ceteris paribus*. Similarly, price reductions in soybeans tend not to stimulate exportation of this product. This is because soy is a basic, non-perishable raw material, difficult to replace because its protein is the only one available in the plant kingdom that has high quality and is easily digestible by the human body, as pointed by Hughes et al. (2011).

Moreover, the governance structure of the supply chain and the market power of the firms that are part of the soybean processing industry can help explain the price inelasticity of this commodity, once part of its exports is made between companies controlled by the same group. In this marketing paradigm, price increases or decreases tend not to change significantly the amount traded. Such price inelasticity results in greater submission of the soybean farmers to the domain of the processing industries and reinforces the theories postulated by Ferguson and Ferguson (1994), Possas (1996) and Pindyck and Rubinfeld (2007). It also indicates the possibility of the exercise of market power via prices by companies such as Bunge S.A., Cargill Agrícola S.A. and other corporations leading the sector.

The coefficient of cross-price elasticity of soybeans supply in relation to the price of soybean meal ($E_{Q_g P_f}$) presented a positive sign, indicating that the increased price of soybean meal tends to result in increased exports of soybeans. So, a 10% rise in the price of soybean meal lagged behind by one period tends to result in an increase of 6.78% in the amount of exports of soybeans, *ceteris paribus*, as can be seen in (6).

$$E_{Q_g P_f} = \beta_3 \frac{\overline{PXF}_{-1}}{\overline{QTXG}} = 22.055{,}86 \frac{316{,}05}{10.280.953} = 0{,}6780 \quad (6)$$

Economic rationality indicates in the analysis of supply

that price increases of soybean meal would tend to result in decreased exports of soybeans because the profits maximization would be achieved with soybean meal exports. However, soybeans trade is predominantly intra-industry and intra-firm, so that exoneration of Brazilian exports and the incidence of *ad valorem* duties on soybean meal imports lead the companies to maximize profits by producing meal in plants outside the country.

The cross-price elasticity of soybeans supply in relation to the export price of soybean oil ($E_{Q_g P_o}$) was calculated by Equation 7 and the coefficient was at the level of -0.175.

$$E_{Q_g P_o} = \beta_4 \frac{\overline{\text{PXO}}}{\overline{\text{QTXG}}} = -2.350{,}865 \frac{768{,}19}{10.280.953} = -0{,}1757 \quad (7)$$

This result can be explained by the fact that price rises of soybean oil may result in an increased demand for palm oil and, consequently, in a reduced demand for soybeans. It is worth noting that the coefficients of cross-price elasticity of soybean meal and oil in relation to the Brazilian soybeans exports showed different signs and magnitudes, which corroborates the fact that even though deriving from the same raw material and produced by the same companies they are traded in markets with different characteristics.

The effect of fluctuations in the real exchange rate was also estimated by the econometric model and the results show that exchange rate depreciations contribute to increased exports, *ceteris paribus* (Equation 8).

$$E_{Q_g TRC_{R\$xUS\$}} = \beta_5 \frac{\overline{\text{REXCHANGE}}}{\overline{\text{QTXG}}} = 2.902.130 \frac{3{,}29}{10.280.953} = 0{,}9287 \quad (8)$$

In this case, a 10% appreciation of the exchange rate tends to reduce soybean exports in 9.29% *ceteris paribus*. Likewise, a 10% depreciation of the exchange rate tends to result in an increase of 9.29% of the exported volume. This shows the importance of the exchange rate and corroborates the theories postulated by Zini Júnior (1995) and Krugman and Obstfeld (2010) that exchange rate variations produce changes in the purchasing power of products and, therefore, interferes with the exported volume.

Income, as proposed by the authors studied, is also a representative variable in the analysis of trade flows. Thus, the coefficient of elasticity of soybean exports in relation to the domestic income ($E_{Q_g I_d}$) and that of international markets ($E_{Q_g I_a}$) was calculated, as shown in Equations 9 and 10.

$$E_{Q_g I_d} = \beta_6 \frac{\overline{\text{GDPBR}}}{\overline{\text{QTXG}}} = 2.527{,}052 \frac{6.590{,}54}{10.280.953} = 1{,}6200 \quad (9)$$

It was expected negative sign for coefficient $E_{Q_g I_d}$, because as observed by Krugman and Obstfeld (2010), a GDP growth in the exporting country tends to result in an increased domestic consumption, including imports. But the opposite sign and the magnitude of the same can be accepted, once the companies that are part of Brazil' soybean processing industry are mostly held by groups that also have subsidiaries in other markets and, regardless the domestic conditions of the Brazilian economy, they need to supply the industries located in other countries.

On the other hand, the coefficient ($E_{Q_g I_a}$) confirmed the theoretical postulates and empirical studies conducted by Goldstein and Khan (1978), Zini Júnior (1988; 1995), Dornbusch and Fischer (1994), Castro and Cavalcanti (1997), Cavalcanti and Ribeiro (1998), Onunkwo and Epperson (1999), Santana (2002) and Barros et al. (2002).

$$E_{Q_g I_a} = \beta_7 \frac{\overline{\text{GDPAS}}}{\overline{\text{QTXG}}} = 5.206{,}334 \frac{2.666{,}97}{10.280.953} = 1{,}3506 \quad (10)$$

This shows that Brazil's exports of soybeans are elastic to the Asian income. In this respect for every 10% increase in the Asian income, the trend is an increase of 13.51% in Brazilian exports of soybeans, *ceteris paribus*. The opposite is also true. However, this situation requires a careful analysis, especially the planning of policies to reduce the dependence on this market, since in times of crisis trade tensions or something with Asia, or even the development of technologies that would enable to add part of the African land to soybean production, the Brazilian supply chain would be significantly impacted.

Econometric analysis of Brazil's exports of soybean meal

As occurred in the estimation of the exports model for soybeans, the econometric estimation for the analysis of the soybean meal market aggregated historical series in the 1980-2000 period. The indirect influence of the European Gross Domestic Product (a key consumer market for soybean meal) and the corn price (product that complements soybean meal in the production of animal feeds) was calculated, as well as the direct influences of variables PXF, PXF^2, PXG, PXO, $REXCHANGE^2$, GDPBR, GDPAS.

The J Statistics at the level of 1.14 and the P-value (J Statistics) of 0.56 confirm the orthogonality of the parameters. The adjusted R-squared indicates that 75.96% of the variations in soybean exports are directly explained by changes in the prices of soybean meal, soybeans and soybean oil, the exchange rate and the GDP *per capita* in Brazil and Asia, and indirectly by

variations in the GDP *per capita* in Europe and the international price of corn.

All estimated parameters, except for that associated with the Brazilian income (GDPBR), are statistically different from zero at the levels of 1%, 5% or 10. The results are shown in Table 2. The coefficient of price elasticity of supply of soybean meal (E_{P_f}) presented a sign consistent with the economic theory and indicates that exports of soybean meals are price inelastic. Thus, for every 10% rise in the meal price, a reduction of 6.56% is expected in soybean meals exports, *ceteris paribus*.

$$\varepsilon_p = \frac{dQ}{dP} * \frac{\overline{Px}}{\overline{Qx}} = (b + 2c\overline{Px}) * \frac{\overline{Px}}{\overline{Qx}} = -39.409,11 + 2 * 28,71 * 324,65 * \frac{324,65}{10.280.953} = -0,6567 \quad (11)$$

This result is attributed to the unavailability of close substitutes for soybean meal in animal feeds. Thus, as soybean meal is essential to feed the total swine, poultry and dairy cattle herds in Europe and Asia, it ensures the consumption of the product in the short and medium term even at high prices.

For the processing industry, the low elasticity means a possibility of price increases and a consequent increase in profits, because trade is made in an oligopolized market protected by barriers to entry. However, exports of soybean meal are traded by firms of the soybean processing industry and animal feed industries directly linked to the production of meat, dairy products and eggs, which results in an oligopolistic competition because it puts face to face two strong segments of the national and international agribusiness.

The coefficient of cross-price elasticity of soybean meal in relation to the price of soybeans ($E_{Q_f P_g}$) was positive and at the level of 0.8817. This shows that for every 10% rise in the soybeans price, exports of meal tend to increase 8.81%, *ceteris paribus* (Equation 12).

$$E_{Q_f P_g} = \beta_3 \frac{\overline{PXG}}{\overline{QTXF}} = 23.798,57 \frac{384,43}{10.376.291,07} = 0,8817 \quad (12)$$

The result $E_{Q_f P_g} > 0$ indicates a substitutability relationship between Brazilian exports of soybeans and soybean meal. The effect obtained by the econometric model is consistent with what is observed in the international market because increases in the exports price of soybeans tend to result in an increase of the production costs of soybean meal produced in industrial plants located outside Brazil. This result complements the analysis of the cross elasticity relationship of exports of soybeans with the price of soybean meal (Equation 6).

The cross relationships between soybean meal and oil were investigated by the cross-price elasticity of the demand of soybean meal in relation to the price of oil ($E_{Q_f P_o}$). The coefficient sign was consistent with the economic theory once the negative sign associated with the coefficient indicates complementarity relation. Such relationship exists especially on the side of supply, once the production of meal and oil is inseparable (Equation 13).

$$E_{Q_f P_o} = \beta_4 \frac{\overline{PXO}}{\overline{QTXF}} = -4.823,505 \frac{791,75}{10.253.892,27} = -0,3724 \quad (13)$$

It is estimated that a 10% reduction in the price of soybean oil, *ceteris paribus*, tends to result in a rise of 3.72% in soybean meal exports. However, it is important to note that even though derived from the same raw material, meal and oil are sold in markets with distinct characteristics: the first is mostly sold to the animal feed industry and does not have close substitutes in the quantity demanded by the market; the latter is mostly consumed by human populations and can be easily substituted with palm, sunflower, rice, corn, canola oils, among others.

The effects caused by variations in the exchange rate of Brazilian exports of soybean meal were analyzed by the coefficient of cross elasticity of the supply of soybean meal in relation to the exchange rate ($E_{Q_g TRC_{R\$xUS\$}}$). The conclusion reached is that the coefficient sign is consistent with theory, but the exchange rate changes tend not to interfere significantly in exports, *ceteris paribus*, as can be seen in Equation 14.

$$E_{Q_f TRC_{R\$xUS\$}} = \beta_5 \frac{\overline{REXCHANGE}}{\overline{QTXF}} = 54.555,23 \frac{3,30}{10.253.892,27} = 0,0176 \quad (14)$$

The coefficient of elasticity of supply of soybean meal exports in relation to the domestic income ($E_{Q_f I_d}$) did not present statistical significance, so it was not analyzed.

The income of consumer markets, taken directly from the Asian GDP and indirectly from the European GDP, indicated a sign consistent with the theory, corroborating the assumptions and results found by Goldstein and Khan (1978), Zini Júnior (1988, 1995), Dornbusch and Fischer (1994), Castro and Cavalcanti (1997), Cavalcanti and Ribeiro (1998), Onunkwo and Epperson (1999), Santana (2002) and Barros et al. (2002). Equation 15 indicates that for a 10% growth in the Asian GDP, a 9.11% increase in the Brazilian exports of soybean meal is estimated, *ceteris paribus*. The opposite is also true.

$$E_{Q_f I_a} = \beta_7 \frac{\overline{GDPAS}}{\overline{QTXF}} = 3.579,755 \frac{2.609,10}{10.253.892,27} = 0,9109 \quad (15)$$

Table 2. Regression to explain export of soybean meal from Brazil.

Variable	Coefficient	Std. Error	t-Statistic	Prob.
C	11.165.955.00	2.172.628.00	5.1394	0
PXF	-39.409.12	16.803.71	-2.3453	0.028
PXF²	28.72	14.51	1.9786	0.06
PXG	23.798.57	12.244.68	1.9436	0.0643
PXO	-4.823.51	2.613.15	-1.8459	0.0778
REXCHANGE²	54.555.23	29.700.98	1.8368	0.0792
GDPBR	-1.033.63	806.3	-1.2819	0.2126
GDPAS	3.579.76	1.306.30	2.7403	0.0117
R-squared	0.8157	Mean dependent variable		10.253.892.00
Adjusted R-squared	0.7597	S.D. dependent variable		2.274.299.00
S.E. of regression	1.115.081.00	Sum squared resid		2.86E+13
Durbin-Watson stat	1.4908	J-statistic		1.1401
Instrument rank	10	Prob(J-statistic)		0.5654

In this context, it can be seen that the increase of the international income contributed to the expansion of Brazil's exports of soybean meal. Likewise, the coefficient also captured the effects of the increase of income and demand of meats (chicken and pork), milk and eggs, because soybean meal is an input for these segments.

Econometric analysis of Brazil's exports of soybean oil

Many factors have contributed to the growing world supply of edible oils in the recent decades, such as the significant population growth (Brum, 1993, 2002), the increased income in the developed and developing economies (Giordano, 1999) and the adoption of new technologies, which resulted in a larger supply of raw materials and higher yields in the process of extraction of soybean oil (Thomas, 2003).

In this process, the growing production of palm, soybean, canola and sunflower oils began to account for most of the additional supply of vegetable oils (FAO, 2012), but unlike the soybeans and meal markets, soybean oil trade occurs in an environment with close substitutes.

To examine the order of integration and the existence of co-integration between these markets, the variables relating to the average world price of palm oil exports (PWPALMOIL), average world price of soybean oil exports (PWSOYOIL), average world price of canola oil (PWCANOLAOIL) and the average world price of sunflower oil exports (PWSUNFLOWEROIL) were submitted to the Augmented Dickey-Fuller (ADF) unit root test and to Johansen's (1988) co-integration test.

The results of the ADF unit root test showed that all variables are first-order integrated, with intercept. Johansen's co-integration test (Table 3) indicates the existence of long-term relationships between the palm, soybean, canola and sunflower oil markets, because the trace test indicated four co-integrating vectors at 5% probability.

Thus, the null hypothesis of non-integration between the series is rejected and the alternative hypothesis is accepted, and the hypothesis that the prices and quantities of soybean, palm, canola and sunflower oils are defined in a same market is confirmed. So, part of Brazil's exports of soybean oil is determined by the market conditions for vegetable oils. Therefore, the analysis of Brazil's exports of soybean oil should include, in addition to the soybean oil price, exchange rate, domestic income and international income, the price of the main substitute products.

Based on the Generalized Method of Moments (GMM), with information of the 1980-2010 period, an equation was estimated to examine Brazil's exports of soybean oil (QTXO). The data were weighed by the HAC matrix (Bartlett kernel, Newey-West with fixed bandwidth = 4,0000). The instrumental matrix aggregated the variables PWSOYOIL, REXCHANGE, GDPBR, GDPEU, GDPAS, PWPALMOIL, PWCANOLAOIL and PWSUNFLOWEROIL. The results are displayed in Table 4.

The J Statistics, at the level of 1.77 and P-value (J Statistics) of 0.41, confirms the parameters orthogonality and model consistency. The coefficient of determination of adjusted R-squared indicates that 80% of the variations in Brazil's exported quantities of soybean oil are directly explained by variations in the international price of soybean oil, real exchange rate, Brazil income, Europe income, the price of substitute products (palm, canola and sunflower oils), and indirectly by the Asian market income.

All parameters were statistically significant at 1% probability, except those associated with the variables REXCHANGE and PWSUNFLOWEROIL, which were statistically different from zero at 5% probability.

Table 3. Johansen Cointegration Test to assess long-term relationship in the vegetable oil markets.

Sample (adjusted): 1964 2010				
Included observations: 47 after adjustments				
Trend assumption: Linear deterministic trend				
Series: D(SOJA) D(PALMA) D(CANOLA) D(GIRASSOL)				
Lags interval (in first differences): 1 to 1				
Unrestricted Cointegration Rank Test (Trace)				
Hypothesized No. of CE(s)	**Eigenvalue λi**	**Trace Statistic**	**0.05 Critical Value**	**Prob.****
None *	0.820807	157.515	47.85613	0.0000
At most 1 *	0.47316	76.7083	29.79707	0.0000
At most 2 *	0.44517	46.58795	15.49471	0.0000
At most 3 *	0.331113	18.90056	3.841466	0.0000
Trace test indicates 4 cointegrating eqn(s) at the 0.05 level				
* denotes rejection of the hypothesis at the 0.05 level				
**MacKinnon-Haug-Michelis (1999) p-values				

Unrestricted Cointegration Rank Test (Maximum Eigenvalue)				
Hypothesized No. of CE(s)	Eigenvalue λi	Max-Eigen Statistic	0.05 Critical Value	Prob.**
None *	0.820807	80.80666	27.58434	0.0000
At most 1 *	0.47316	30.12035	21.13162	0.0021
At most 2 *	0.44517	27.68738	14.2646	0.0002
At most 3 *	0.331113	18.90056	3.841466	0.0000
Max-eigenvalue test indicates 4 cointegrating eqn(s) at the 0.05 level				
* denotes rejection of the hypothesis at the 0.05 level				
**MacKinnon-Haug-Michelis (1999) p-values				

The cross-price elasticity coefficient of Brazil's exports of soybean oil in relation to the international price of soybean oil ($E_{Q_o P_{PMOSOJA}}$) was of -4.28 and shows that the Brazilian exports are extremely elastic in relation to the international prices (Equation 16).

$$E_{Q_o P_{PMOSOJA}} = \beta_1 \frac{\overline{\text{PWSOYOIL}}}{\overline{\text{QTXO}}} = -6.955{,}622 \frac{849{,}74}{1.381.182{,}22} = -4{,}28 \quad (16)$$

In this context, for every 1% rise in the international price of soybean oil it is expected a reduction of 4.28% in the amount exported, *ceteris paribus*. This result is explained by the high competition in the sector, once palm, canola, sunflower, rice, corn, olive oils and others have the same physical, chemical and nutritional quality standards of, or even higher than, those found in soybean oil, which makes these products perfect substitutes. Thus, as the soybean oil price rises, the expectation is that the reduced consumption of this good is replaced by an increased consumption of substitutes.

The elasticity calculated by Equations 17, 18 and 19 allow to refine this analysis since all coefficients showed positive signs, indicating a substitutability relationship, as demonstrated by Miller (1981), Varian (2006) and Santana and Ribeiro (2008).

$$\text{E}_{\text{Q}_o P_{palma}} = \beta_5 \frac{\overline{\text{PWPALMOIL}}}{\overline{\text{QTXO}}} = 1.353{,}102 \frac{733{,}43}{1.381.182{,}22} = 0{,}72 \quad (17)$$

$$\text{E}_{\text{Q}_o \text{P}_{canola}} = \beta_6 \frac{\overline{\text{PWCANOLAOIL}}}{\overline{\text{QTXO}}} = 5.051{,}924 \frac{913{,}41}{1.381.182{,}22} = 3{,}34 \quad (18)$$

$$\text{E}_{\text{Q}_o \text{P}_{girassol}} = \beta_7 \frac{\overline{\text{PWSUNFLOWEROIL}}}{\overline{\text{QTXO}}} = 1.155{,}094 \frac{951{,}02}{1.381.182{,}22} = 0{,}80 \quad (19)$$

In particular, the substitutability of canola oil in relation to the soybean oil stands out, since the elasticity coefficient was at 3.34, indicating that for every 1% increase in the canola oil price, the trend is an increase of 3.34% in Brazil's exports of soybean oil, *ceteris paribus*. The opposite is also reciprocal. But with respect to the changes in the international price of palm and sunflower oils, Brazil's exports of soybean oil were less elastic. Yet, the model corroborates that for every 10% increase in the

Table 4. Regression to explain Brazilian exports of soybean oil.

Variable	Coefficient	Std. Error	t-Statistic	Prob.
C	-522.886.70	536.222.20	-0.9751	0.3396
PWSOYOIL	-6.955.62	1.111.53	-6.2577	0
REXCHANGE	175.521.90	68.758.63	2.5527	0.0178
GDPBR	-315.8	90.57	-3.4869	0.002
GDPEU	136.19	35.23	3.8661	0.0008
PWPALMOIL	1353.1	444.54	3.0438	0.0058
PWCANOLAOIL	5051.92	1.193.15	4.2341	0.0003
PWSUNFLOWEROIL	1.155.09	549.92	2.1004	0.0469
R-squared	0.8467	Mean dependent var		1.381.182.00
Adjusted R-squared	0.8	S.D. dependent var		656.195.70
S.E. of regression	293.441.30	Sum squared resid		1.98E+12
Durbin-Watson statistic	1.274	J-statistic		1.7713
Instrument rank	10	Prob(J-statistic)		0.4125

international price of palm oil, a 7.2% increase in Brazil's exports of soybean oil is expected, *ceteris paribus*. Also, a 10% increase in the international price of sunflower oil tends to result in 8% increase of Brazil's exports of soybean oil. So, the integration of these markets and its impact on Brazil's exports of this product are reaffirmed.

The degree of interrelation of these markets is also explained by the composition of the international supply of edible oils, where soybean, palm, canola and sunflower oils account for 82% of the supply and are the most consumed oils, mainly in markets such as Brazil, India, China, South Africa, Mexico, among others, whose *per capita* income is not as high as that in the developed countries (FAO, 2012).

The effects of the fluctuations in the exchange rate are demonstrated in Equation 20. It can be seen that the coefficient of cross elasticity of the exported amount of soybean oil in relation to the real exchange rate was positive. The sign is consistent with the theory, once devaluations of the exchange rate, *ceteris paribus*, represent a reduction in the relative prices and purchasing power of dollar, as described by Krugman and Obstfeld (2010).

$$E_{Q_o TRC_{R\$xUS\ \$}} = \beta_2 \frac{\overline{\text{REXCHANGE}}}{\overline{\text{QTXO}}} = 175.521{,}90 \frac{3{,}30}{1.381.182{,}22} = 0{,}42 \quad (20)$$

Thus, the results indicate that exchange rate fluctuations tend to result in less than proportional fluctuations in Brazil's exports of soybean oil. Under this perspective, for a10% devaluation on the exchange rate, a 4.2% growth is expected in the exports of soybean oil.

In contrast, the impact of the domestic income is greater once the coefficient of cross elasticity of the exports of soybean oil in relation to the domestic income ($E_{Q_o I_d}$) was of -1.49 (Equation 21). Both the sign and the magnitude are in agreement with the theory, once the increase in domestic income represents greater purchasing power and domestic consumption of soybean oil derivatives, e.g., margarines, mayonnaise, dressings, breads, sweets, candies, chocolates, among other pharmaceutical, industrial and medical products. Under this perspective, for every 1% rise in Brazil's GDP, it is expected a 1.49% reduction in the exports of soybean oil, *ceteris paribus* and vice-versa.

$$E_{Q_o I_d} = \beta_3 \frac{\overline{\text{GDPBR}}}{\overline{\text{QTXO}}} = -315{,}7994 \frac{6.495{,}55}{1.381.182{,}22} = -1{,}49 \quad (21)$$

This coefficient confirms the importance of the domestic market for the industry of vegetable oils and derivatives and, indirectly, for the production of soybeans and soybean meal, demystifying the common sense that soybean is only an export product. On the other hand, the coefficient of cross elasticity of soybean oil exports in relation to the European income ($E_{Q_o I_e}$) was of 1.85, which indicates the importance of the European market for Brazilian exports of soybean oil and confirms the postulates of Goldstein and Khan (1978), Zini Júnior (1988), Dornbusch and Fischer (1994), Zini Júnior (1995), Santana (2002), Castro and Cavalcanti (1997), Cavalcanti and Ribeiro (1998), Onunkwo and Epperson (1999), Barros et al. (2002) and Santana (2002) that the domestic and international income impact trade in a different manner.

$$E_{Q_o I_e} = \beta_4 \frac{\overline{\text{GDPEU}}}{\overline{\text{QTXO}}} = 136{,}1875 \frac{18.734{,}07}{1.381.182{,}22} = 1{,}85 \quad (22)$$

In this sense, the 10% increase in Europe's *per capita* income tends to result in a 18.5% growth in Brazil's exports of soybean oil, *ceteris paribus*. These results are consistent with the current conditions, in which the major

European importers (Holland, Germany and Spain) do not impose tariffs or quotas for soybean oil imported from Brazil (BRASIL, 2012).

Finally, the analyses allow stating that as a function of the competition by other edible oils, especially palm, sunflower and canola oils, the soybean processing industry cannot project its market dominance to the segment of vegetable oils. This situation explains the *filière* strategy of investments in "key activities" at the links directly upstream and downstream in the soybean supply chain. This was the alternative found to determine governance and, from it, create conditions for designing a scenario to enable the sale of soybeans and oil.

Thus, the high competition in the supply of vegetable oils tends to result in the submission of soybean producers to the interests of the industry, especially those who are unassisted by government credit lines and depend on trading companies to survive.

Therefore, this study neither minimizes the importance of the industry to agriculture nor the importance of the grains processing industry for the expansion of the area planted and increased soybean yields in Brazil, but underlines that the markets are ruled by a small number of corporations (Bunge Alimentos S.A., Cargill Agrícola S.A., ADM do Brasil Ltda., Louis Dreyfus Commodities Brasil Ltda., and Multigrain S.A., among others), and emphasizes the importance of the government in offering credit lines for investment, expenditures and marketing, as well as effective efforts to support fair competition. Hence, the market relationships throughout the soybean supply chain will be fairer and more balanced.

DISCUSSION

The econometric analysis allowed quantifying the importance of price, fluctuations in the exchange rate and in the domestic and international income for the Brazilian exports of soybeans, soybean meal and oil.

The results showed that Brazil's exports of soybeans are price inelastic, the growth of exports is directly and positively associated with the international income growth, especially in Asia, and that exports fluctuations are also influenced, to a lesser degree, by fluctuations in the exchange rate.

Similar results were found in the analysis of exports of soybean meal, in which it was observed price inelasticity for soybean meal, a substitutability relationship between soybean meal and soybeans in exports, the reduced effect of exchange rate fluctuations and the importance of the Asian income.

But in the analysis of exports of soybean oil, a long-term relationship in the markets of soybean oil, palm oil, canola oil and sunflower oil was found by four co-integrating vectors pointed by the Johansen's co-integration test. This implies the existence of a dynamic and integrated movement in the global key oil producing markets. This finding is confirmed by the econometric estimation of the soybean oil export function, in which a high price elasticity of this product and cross elasticity of this product with other oils, particularly canola oil, were found. As observed for the markets of soybeans and meal, the international income is an important determinant of Brazil's exports, and the exchange rate does not play a major part in this process.

Therefore, by trading inelastic products and protected by barriers at entry, the soybean processing industry can shift the market breakeven point to a position that maximizes the economic outcomes, that is, the dominant firms can project their position to attract the surplus produced by soybean farmers.

So, it is clear that a significant portion of the transnational corporations' competitiveness in the soybean business results from strategies of positioning themselves at the links directly upstream and downstream in the supply chain, Brazil's large domestic and international market share and the low price elasticity of soybeans and soybean meal.

Thus, the authors can conclude that soybean growers are in a weak situation, because they demand inputs from an oligopolistic market, offer their production to an oligopsonized market, and the prices for their commodity are inelastic.

Finally, it is suggested that the creation of mechanisms to reduce the exposure of soybean growers to the market power exerted by big companies, and the creation of sectorial policies to foster competition, without which the soybean supply chain would be at risk of being consolidated as a mere instrument for capital accumulation by big transnational corporations.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Effects of hydrogel and nitrogen fertilization on the production of arugula in successive crops

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Appropriate management techniques, such as fertilization programs and the use of technology in the production process, have been employed to meet the demand for vegetables year-round. Because vegetables require a considerable amount of water for their development, hydrogels can guarantee the supply of water in regions with water deficits. The aim of this study was to evaluate the use of hydrogel and nitrogen fertilizer on the development and productivity of arugula cv. Cultivada in two successive crops. The experiment was conducted at the State University of Mato Grosso do Sul (Universidade Estadual de Mato Grosso do Sul – UEMS), Aquidauana University Unit, in the state of Mato Grosso do Sul, Brazil, from April to July 2011. The experimental design consisted of randomized blocks with a 2×5×2 factorial scheme, with and without application of hydrogel (polymer), five different doses of nitrogen (0, 60, 120, 180 and 240 kg ha^{-1}) and two successive crops of arugula, with four replications. The gel was applied to the first crop, and the residual effect on the second crop was evaluated. The results show that the hydrogel had no effect on the fresh and dry mass of arugula, regardless of the cultivation period. The use of the N fertilizer significantly affects the development of arugula, as evidenced by a linear increase in the shoot dry mass, number of leaves and leaf area. The application of N influenced the components of production and productivity of arugula.

Key words: *Eruca sativa* Miller, brassica, hydrogel, nitrogen.

INTRODUCTION

The arugula is a herbaceous leafy vegetable belonging to the family Brassicaceae, a large family with more than three thousand species. Its name comes from the Italian "ruccola", and its center of origin is the Mediterranean region in southern Europe and western Asia. The crop has a short growth cycle of between 30 and 40 days and prefers mild temperatures for vegetative development, but it has been cultivated in various regions throughout

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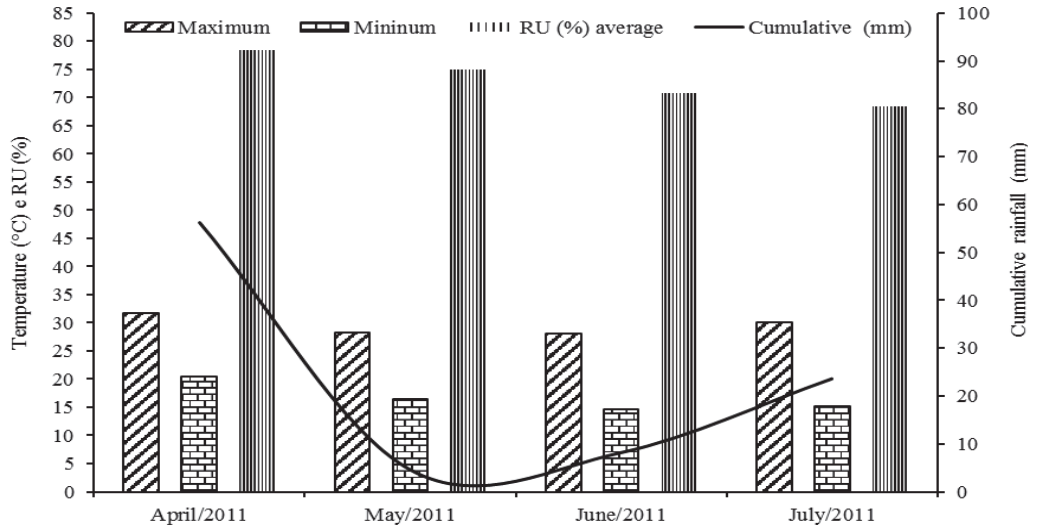

Figure 1. Values of temperature (°C), mean relative air humidity-HR (%) and cumulative rainfall (mm) during the experimental period. Source: Station INMET (2011).

the year (Camargo, 1992; Filgueira, 2012). Arugula has great value for human nutrition and health, being one of the most nutritious vegetables; its leaves are rich in vitamins A and C and in minerals, especially potassium, calcium, iron and sulfur (Trani and Passos, 1998), in addition to being an excellent appetite stimulant as well as conferring anti-inflammatory and detoxifying effects to the body (Linhares et al., 2007).

Because vegetables require a considerable amount of water for their development, the hydro-retention gel can guarantee the supply of water to plants in regions with water deficits. During the formation of the gel, the polymer absorbs water and increases in volume in up to 200%, improving the availability of water by reducing loss through evaporation and subsequently gradually releasing the water to nearby plants. The addition of hydrogels to the soil increases soil water retention, reduces the leaching of nutrients and improves aeration and the cation exchange capacity (Azevedo et al., 2002). Vale et al. (2006), Oliveira et al. (2004) report that hydrogels can be used to stimulate the growth of trees in greenhouses and in reforestation of degraded areas, also minimizing the possible effects of dry spells during the implementation phase of the crop.

According to Sita et al. (2005), the studies on the interaction among hydrogels, substrates and fertilizers are few and inconclusive, and further studies on the subject are required. The same authors reported that the polymer adversely affects the absorption of nutrients and biomass production but contributes substantially to increase K in substrates consisting of soil with organic compost, soil with tobacco waste compost or soil alone. They also observed inverse relationships between polymer doses and biomass and between polymer application and the uptake of K, Ca and Mg, independent of the substrate and of the source of nitrogen and potassium fertilizers used.

Because arugula is a leafy vegetable, nitrogen fertilization is important (Purquerio et al., 2007). Recommendations for application of N in arugula are in the range of 40 kg N ha^{-1} at sowing and 90-150 kg N ha^{-1} as topdressing (Trani and Raij, 1996). Nitrogen has structural functions in plants, as a constituent of amino acids, proteins, nitrogenous bases, many enzymes and energy transfer components, such as chlorophyll, ADP and ATP, and it also plays a role in the processes of ion absorption, photosynthesis, respiration, cell division and cell differentiation (Malavolta et al., 1997).

This study aimed to evaluate the use of the hydrogel and various doses of nitrogen on the development and productivity of arugula cv. Cultivada in two successive crops.

MATERIALS AND METHODS

The experiment was carried out at the State University of Mato Grosso do Sul (Universidade Estadual de Mato Grosso do Sul - UEMS), in the experimental area of the Aquidauana University Unit in the state of Mato Grosso do Sul, Brazil, at geographic coordinates 20º 20' South, 55º 48' West and at a mean elevation of 174 m. The experiment was performed from April to July 2011. The climate in the region belongs to the Aw category in the Köppen classification, defined as a sub-humid warm tropical climate; the average annual rainfall is 1200 mm, and there is a rainy season in the summer and a dry season in the winter.

Maximum temperatures can exceed 40ºC in the summer, and minimum temperatures can approach 5ºC in the winter. The climate data for the period of this experiment are shown in Figure 1. The soil of the experimental area is classified as an Oxisol (very clayed), and deep (Embrapa, 2006). Analysis of the soil in the experimental area showed the following chemical composition: pH ($CaCl_2$) = 5.5; H + Al = 21 $mmol_c$ dm^{-3}; Ca = 62 $mmol_c$ dm^{-3}; Mg = 22 $mmol_c$ dm^{-3};

Table 1. Mean values of shoot fresh mass (SFM), shoot dry mass (SDM), root fresh mass (RFM) and root dry mass (RDM) of arugula on functions of the application of the hydrogel on successive crops.

Treatments	SFM (g)		SDM (g)	
	1º crops	2º crops	1º crops	2º crops
with gel	81.94^{aA}	76.93^{aA}	6.14^{aA}	5.17^{aA}
Without gel	74.30^{aA}	76.43^{aA}	5.40^{aA}	5.55^{aA}
CV (%)	25.43	32.08	28.24	29.31
Treatments	**RFM(g)**		**RDM(g)**	
	1ºcrops	**2ºcrops**	**1ºcrops**	**2ºcrops**
with gel	2.47b^{A}	5.38^{aA}	0.41b^{A}	0.62^{aA}
Without gel	2.24b^{A}	5.36^{aA}	0.35b^{A}	0.64^{aA}
CV (%)	25.21	19.64	33.27	19.87

Means followed by the same lowercase letter in the same row and the same uppercase letter in the same column, for each factor studied, do not differ by the Tukey test at 5% probability.

P (resin) = 68 mg dm^{-3}; K = 7.0 $mmol_c$ dm^{-3}; organic matter = 52 g dm^{-3}; CEC = 112 $mmol_c$ dm^{-3}; %V = 81; Cu (DTPA) = 8.0 mg dm^{-3}, Fe (DTPA) = 75.0 mg dm^{-3}, Mn (DTPA) = 20.6 mg dm^{-3}, Zn (DTPA) = 0.9 mg dm^{-3} and B (hot water) = 0.32 mg dm^{-3}. The soil texture presents 56, 29 and 15% of clay, silt and sand, respectively. The soil porosity is 49% to field capacity and permanent wilting point of 32.3 and 24.8% respectively.

A randomized block design was used for the experiment, in a 2×5×2 factorial scheme, with and without application of the Forth Gel® hydrogel (4.0 g of product m^{-2}), five doses of N (0, 60, 120, 180 and 240 kg ha^{-1}) and two successive crops of arugula, with four replications. The gel was applied to the first crop (C1), and the residual effect on the second crop (C2) was evaluated. The gel is hidroretentor consisting of potassium polyacrylate copolymer, physical characteristics of white crystals of different particle sizes for each specific application condition and can absorb an average of 200 to 400 times its mass, increasing its volume until 100 times.

The arugula cv. Cultivada was planted in four beds of 12×0.8×0.2 m, corresponding to length, width and height. The parcels consisted of four rows of 1.0 m in length spaced 0.15 m apart. The useful area consisted of the two central rows of each parcel. The base fertilization was performed 20 days before planting on the total area, using 200 kg ha^{-1} in the proportions 4-20-20 (N, K, P). The gel was applied by mixing 25 g of the polymer in a portion of soil taken from each parcel to a depth of 0.08 m, and the mixture was then returned and homogenized in the corresponding parcel. Sowing was performed manually in furrows of 0.01 m deep on May 11, 2011 for the first crop and on June 17, 2011 for the second crop. The thinning was performed 9 and 11 days after sowing (DAS) for the first and second crops, respectively, leaving the plants spaced 0.05 m apart.

For the nitrogen fertilization, urea was used as the source because 45% of its nitrogen is accessible. The doses were divided and applied over the soil between crop rows at a distance of approximately 0.05 m from the plant at 7, 14 and 21 days after emergence (DAE), respectively. To avoid competition for water and nutrients, the control of invasive plants was performed manually when needed, and pest control was performed by applying insecticides recommended for the crop. Water was supplied by a conventional sprinkler irrigation system on alternating days with the hydrogel supplying the water in the interim.

Plants were harvested 30 days after sowing in the first crop and 42 days after sowing in the second crop. At harvest, evaluations were performed on the height of plants chosen randomly within the useful area of the plot, the number of leaves per plant, and the fresh and dry mass of roots and shoots. To determine the fresh and dry mass, the plants were placed in paper bags and dried in a forced air oven at 65ºC until they reached constant weight (weighed on a digital precision scale); the leaf area was measured with the help of the IMAGE software TOOL®, and the productivity was calculated per hectare.

Data were subjected to analysis of variance (ANOVA), means were compared by the Tukey test at 5% probability for the effect of the application of the hydrogel, and regression analysis was used for the effect of the nitrogen doses. For the difference between crops, a joint analysis was performed. Statistical analyses were processed using the statistical analysis program Sanest.

RESULTS AND DISCUSSION

Based on the results there was no significant interaction effect between hidroretentor gel application (polymer) and nitrogen doses in the culture of the rocket, for any of the parameters. The analysis of the effect of the hydrogel in each arugula cultivation period demonstrated that the mean values of shoot fresh mass (SFM), shoot dry mass (SDM), root fresh mass (RFM) and root dry mass (RDM) did not differ significantly between the presence and absence of gel application (Table 1). These results may be explained by the rainfall (Figure 1) that most likely provided a similar water supply for the plants, especially in the final periods of each cycle, when there is an increase in mass accumulation.

According to Grangeiro et al. (2011), leafy vegetables have an initial slow phase of dry mass accumulation that increases at the end of the growth cycle. Although the amount of water available for the plants is increased with the use of the hydrogel, its time of availability is also important for the plants and is determined by the rate of evaporation from the soil (Akhter et al., 2004); thus, the results obtained in this study may be explained by observing the mean maximum temperatures during the experimental period (Figure 1).

According to Woodhouse and Johnson (1991), the

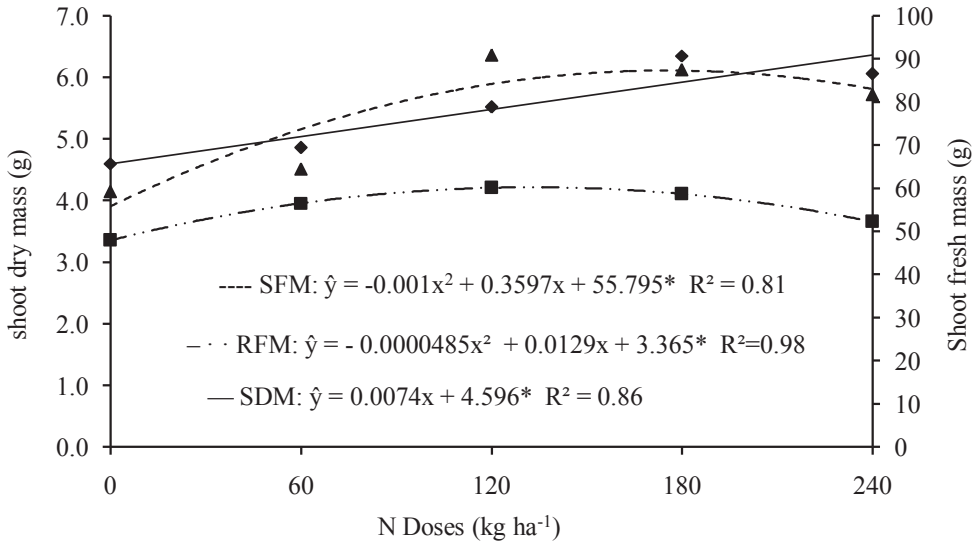

Figure 2. Shoot fresh mass (SFM), shoot dry mass (SDM) and root fresh mass (RFM) as functions of the nitrogen doses in successive crops of arugula. *Significant at p<0.05.

efficiency of water use is not only a measure of how much water is needed to produce one unit of dry mass but is essential for the evaluation of the performance of a polymer in a soil-plant growth system because it includes the evapotranspiration by the plant and evaporation of the soil-polymer mixture.

The results of this study differ from those of Sayed et al. (1991), who evaluated the effect of a hydrogel in the cultivation of various vegetables and found an increase in the fresh and dry mass of plants with the incorporation of the polymer, compared to cultivation without the polymer. Only RFM and RDM differed significantly between the cultivation periods (Table 1); for both parameters, greater root biomasses were obtained when evaluating the residual effect of the hydrogel in the second crop (C2). The arugula grown in soil that received the hydrogel had greater SFM and SDM values in the first crop (C1) than in the second crop, but the difference was not significant.

The nitrogen dosage had a significant effect on SDM, SFM, and RFM, independent of hydrogel application, and an increase in N produced a linear increase in SDM. These results differed from those observed by Purquerio et al. (2007), in which the SDM data for arugula were described by a quadratic polynomial equation.

Regarding fresh mass, there was a quadratic fit of the data to quadratic polynomial regressions with maximum values estimated at 179.8 and 132.9 kg N ha^{-1} for the SFM and RFM, respectively (Figure 2). Ratke et al. (2011), working with N fertilization in the cultivation of arugula, found that the SFM increased quadratically with a maximum value of 600 kg ha^{-1}, which is greater than that obtained in the present study.

When assessing the number of leaves in C1, the plants that were treated with the hydrogel had a greater number of leaves than the plants grown without the polymer, but no difference was observed in C2 (Table 2). The addition of hydropolymers in the soil favors the availability of water, reduces nutrient loss by leaching, improves soil aeration, and thereby accelerates the shoot development of plants (Peterson, 2009). Marques and Bastos (2010) tested various doses of hydrogel in the cultivation of chili and observed that, even with daily irrigation, increasing doses of hydrogel produced a positive linear increase in the number of leaves.

Regarding the cultivation periods, the C1 plants had more leaves than the C2 plants, regardless of the presence of the hydrogel. For the leaf area, only in the second crop was a difference observed when using the hydrogel: plants that received gel treatment had greater leaf area (Table 2). Taiz and Zeiger (2009) explain that leaf expansion is driven by turgidity. When the water content of the plant decreases, the cells contract and loosen the turgor pressure against the cell walls. With a low leaf area, there is less transpiration, and the limited water supply in the soil is conserved for a longer period of time. Thus, the decrease in leaf area can be a defense mechanism against drought. Carvalho et al. (2013) observed that in passion fruit plants, the thickness of the leaves is reduced in seedlings grown without the hydropolymer and that the same effect was observed on specific leaf area, which relates the leaf surface to the weight of the leaf, representing the leaf thickness.

As observed in Table 2, there was no difference in plant height in either crop regarding treatments with and without the hydrogel. In contrast, the plants were significantly taller in C1 than in C2, either with or without application of the gel. Costa et al. (2011) and Dantas and Torres (2010) obtained plants with maximum heights of

Table 2. Mean values of the number of leaves, leaf area, height and productivity of the arugula as functions of the application of the hydrogel in successive crops.

Treatments	Leaf of number		Leaf area (cm²)	
	1º crops	2º crops	1º crops	2º crops
With gel	9.87 bA	12.93aA	295.01^{aA}	277.65^{aA}
Without gel	8.60 bB	13.68aA	246.67^{aA}	230.41^{aB}
CV (%)	14.93	15.43	22.33	14.83
Treatments	**Height (cm)**		**Productivity (kg ha^{-1})**	
	1º crops	1º crops	1º crops	2º crops
with gel	20.06 aA	13.93^{bA}	12682^{aA}	10392^{bA}
Without gel	19.53 aA	13.88^{bA}	12191^{aA}	11055^{aA}
CV (%)	9,54	15.62	18.89	19.72

Means followed by the same lowercase letter in the same row and the same uppercase letter in the same column, for each factor studied, do not differ by the Tukey test at 5% probability.

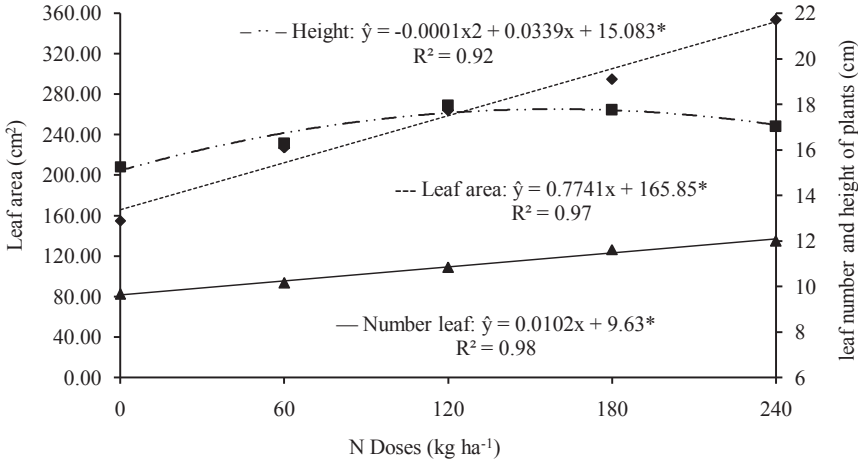

Figure 3. Leaf area, leaf number and height of arugula plants as functions of the N doses in successive crops. *Significant at p<0.05.

27.5 and 21.75 cm at 37 and 36 days after sowing, respectively.

There was no correlation between gel application and plant height as a function of the increasing doses of N (Table 2). Thus, N fertilization can be used without damage to the development and size of arugula. According to Peterson (2009), hydropolymers also have the ability to promote plant growth when nutrients are incorporated into the soil, by releasing the nutrients to the plants when needed. However, in certain situations, the addition of nutrients has shown little effect on plant performance, especially when higher levels of fertilizers and salts are present.

The mean productivity values showed no significant variation between treatments with and without the hydrogel in the two successive crops (Table 2). In the treatment with application of hydrogel, there was higher productivity in C1, whereas without hydrogel application, the productivity levels of the two crops were similar. Regarding productivity, a significant relationship was observed between N doses and the cultivation periods of arugula cv. Cultivada. During the C2 period, there was a climatic change (the mean minimum temperature was 14.7ºC) that caused a delay in germination and a consequent delay in the harvest, resulting in lower values of the variables analyzed.

An example of a difference in productivity achieved during different cultivation periods for the arugula crop was reported by Purquerio et al. (2007), who found that in the summer, high rainfall during the crop cycle and its concentration in short periods of time was detrimental to plants cultivated in the field and, in addition to lower productivity, lower plant quality was also observed. Regarding the N doses, there was a linear increase in both the number of leaves and the leaf area in response to increasing N, and the data were fitted with a positive linear regression (Figure 3). These results differ from those obtained by Purquerio et al. (2007) working with N

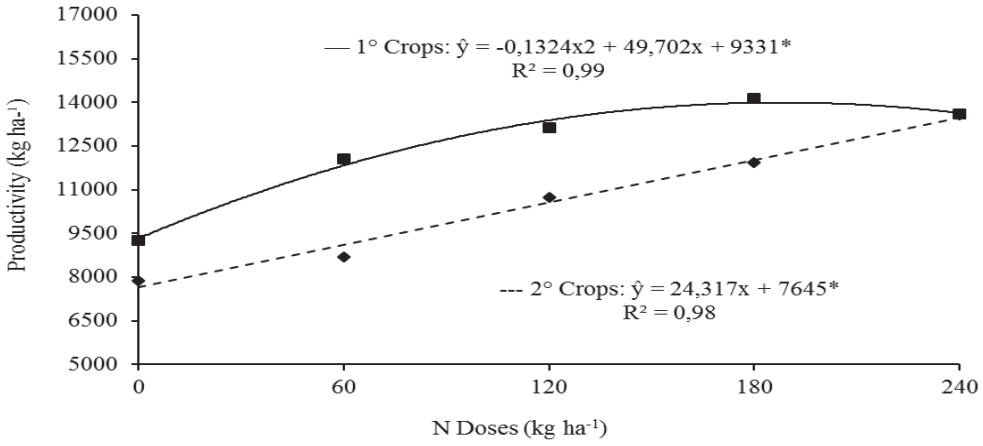

Figure 4. Arugula productivity as a function of N doses in two successive crops. *Significant at p<0.05.

doses on large-leaf arugula, who found that the leaf area values fit a quadratic regression. According to the same authors, there is no standardization for the cultivation of arugula that allows the classification of the leaf area and the mean values of the plant height obtained, which in their analysis were essentially satisfactory. Both wholesalers and consumers prefer packs with large leaves, but it depends on the population of each region.

Figure 3 shows a significant effect of N dose on plant height, but only in the first crop, and the data fit a quadratic polynomial equation, with a maximum value estimated for the dose of 169 kg N ha^{-1}. These results corroborate those of Oliveira et al. (2010), who evaluated two crops and observed differences in plant height and shoot dry mass between the first and second arugula cycles, with the highest mean height in the first crop and the highest SDM in the second.

Regarding productivity, the increasing N doses caused significant variations in the data, which fit a quadratic polynomial equation, with the maximum value estimated at 188 kg N ha^{-1} for the first crop, whereas in the second crop, there was an N dose increase (Figure 4). The estimated dose of N that provided the highest productivity in this study is above the 120 kg ha^{-1} recommended by Trani and Raij (1996) for the cultivation of arugula. These results are consistent with those obtained by Steiner et al. (2011), who observed an increase in the production of rocket with application of nitrogen. However, the results obtained diverge from those reported by Purquerio et al. (2007), who obtained an increase in the productivity of arugula cv. Folha Larga (Large Leaf) up to the estimated dose of 240 kg ha^{-1}.

Conclusions

(1) The results show that the hydrogel had no effect on the fresh and dry mass of arugula, regardless of the cultivation period.
(2) The use of the N fertilizer significantly affects the development of arugula, as evidenced by a linear increase in the shoot dry mass, number of leaves and leaf area.
(3) The application of N influenced the components of production and productivity of arugula.

Conflict of Interest

The authors have not declared any conflict of interest.

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