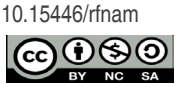
Periodicidad: Cuatrimestral
Vol. 73 No. 3 - 2020

Admitida en las Bases Bibliográficas:
Scopus
Scielo (Scientific Electronic Library Online)
ISI-Scielo Citation Index
REDIB (Red Iberoamericana e innovación y conocimiento científico)
Cabi (www.cabi.org)
EBSCO Host
Google Scholar
DOAJ (Directory of Open Access Journals)
Ulrich's Periodicals Directory (Global Serials Directory)
Redalyc (Red de Revistas Científicas de América Latina, el Caribe, España y Portugal)
Latindex (Sistema Regional de Información en Línea para Revistas Científicas de América Latina, el Caribe, España y Portugal)
ProQuest
Teeal (The Essential Electronic Agricultural Library)
WZB (Berlin Social Science Center)
Cross ref
Cornell University
Field Crop Abstracts
Forestry Abstracts
Plant Breeding Abstracts
Índice Agrícola de América Latina y el Caribe
Índice Bibliográfico Nacional
Colciencias - Publindex

Portada: "Nuestro campo" Alba Nelly Cadena Chamorro
Contraportada: Klara Torres Restrepo
Dirección postal: Apartado Aéreo 568, Medellín, Colombia
Dirección electrónica: rfnagron_med@unal.edu.co
Página Web: http://www.revistas.unal.edu.co/index.php/refame
Teléfono: (*4) 430 90 06; Fax: (* 4) 230 04 20
Diagramación: Miryam Ospina Ocampo
Marcación: LandSoft S.A.
Diseño e Impresión: Prográficas - Proyecciones Gráficas
Primera edición: Año 1939
ISSN: 0304-2847
ISSN formato web: 2248-7026
doi: 10.15446/rfnam



Licencia Ministerio de Gobierno: 275/64

EVALUADORES

El Comité Editorial dentro de sus políticas, envía los artículos a especialistas, con el fin de que sean revisados. Sus observaciones en adición a las que hacen los editores, contribuyen a la obtención de una publicación de reconocida calidad en el ámbito de las Ciencias Agrarias. Sus nombres son mencionados como una expresión de agradecimiento.

Ady Giordano. Pontificia Universidad Católica de Chile. Santiago de Chile, Chile. agiordano@uc.cl

Armando Sterling. Instituto Amazónico de Investigaciones Científicas–Sinchi. Florencia, Colombia. asterling@sinchi.org.co

Aurelio San Martín. Universidad de Chile. Santiago, Chile. asmbarr@gmail.com.

Carlos Enrique Castilla. Agrosavia. Nariño, Colombia. ccastilla@agrosavia.co

Dario Antonio Castañeda. Universidad Nacional de Colombia - Sede Medellín. Medellín, Colombia. dacasta4@unal.unal.edu.co

Edislon León Moreno Cardenas. Universidad Nacional de Colombia - Sede Medellín. Medellín, Colombia. elmorenoc@unal.edu.co

Eduardo L. Jerez Monpie. Instituto Nacional de Ciencias Agrícola (INCA). La Habana, Cuba. ejerez@inca.edu.cu

Edwin Castro Rincon. Agrosavia. Medellín Colombia. ecastro@corpoica.org.co

Erasto Sotelo Ruiz. Colegio de Postgraduado -UNAL-Sede Bogotá. Bogota, Colombia. igomezm@unal.edu.co

Ervin Humprey Durán Bautista. Universidad de Amazonia. Florencia, Colombia. ervinduranb@gmail.com

Fanor Casierra Posada. Univeresidad Pedagógica y Tecnologica de Colombia. Tunja, Colombia. fanor.casierra@uptc.edu.co

Héctor Suarez Mahecha. Universidad Nacional de Colombia - Sede Bogotá. Bogota, Colombia. hsuarezm@unal.edu.co

Hernán Gonzalez Osorio. Cenicafé. Chinchiná, Colombia. Hernan.Gonzalez@cafedecolombia.com

Jaime Espinosa Tason. Instituto de Investigación Agropecuaria de Panamá. Panamá, Panamá. j.espinosa.idiap@gmail.com

Jorge Bernal Estrada. Agrosavia Medellín, Colombia. jbernal@agrosavia.co

José Régulo Cartagena. Universidad Nacional de Colombia - Sede Medellín. Medellín, Colombia. jrcartag@unal.edu.co

Juan F. Seminario Cunya. Universidad Nacional de Cajamarca. Cajamarca, Perú. jfseminario@yahoo.es

Karina A. Ojeda. Universidad de Cartagena. Cartagena, Colombia. kojedad@unicartagena.edu.co

Katerine Barragán Viloria. Universidad de Sucre. Sincelejo, Colombia. barraganviloria@gmail.com

Lilia Alcaraz Meléndez. Centro de Investigaciones Biológicas del Noroeste. La Paz, Mexico. lalcaraz04@cibnor.mx

Lourdes Orejuela. Universidad Francisco de Quito. Quito, Ecuador. lorejuela@usfq.edu.ec

Luz Yineth Ortiz Rojas. Universidad Francisco de Paula Santander. Cúcuta, Colombia. luzyinethor@ufps.edu.co

Mario Antonio Sandoval. Universidad de Concepcion. Concepción, Chile. masandov@udec.cl

Oscar Balochhi L. Universidad Austral de Chile. Valdivia, Chile. obalocch@uach.cl

Otavio Bagioto Rossato. Instituto Federal Catarinense. Blumenau, Brasil. otavio.rossato@ifc.edu.br

Paulo Roberto Ribeiro Mesquita. Empresa Baiana de Desenvolvimento Agrícola S.A. Salvador de Bahía, Brasil. prrmesquita@gmail.com

Rey Gaston Loor Solórzano. Instituto Nacional de Investigaciones Agropecuarias. Quevedo, Ecuador. rey.loor@iniap.gob.ec

Roberto González Tenorio. Universidad Autónoma del Estado de Hidalgo. Pachuca de Soto, Mexico. rbtogzt@hotmail.com

Silvia Valencia. Escuela Politécnica Nacional. Quito, Ecuador. silvia.valencia@epn.edu.ec

Walter Antonio Roman Junior. Universidade Comunitária da Região de Chapecó. Chapecó, Brasil. romanwa@unochapeco.edu.br

EDITORIAL

Medellín, mayo de 2020

Declaración sobre la pandemia del COVID-19 y el agro colombiano

El país se halla en una crisis súbita sin precedentes, cuyo alcance global reconfigurará, sin lugar a duda y de manera significativa, el mundo tal y como lo conocemos hoy, lo cual podría representar para cada país una oportunidad para reinventarse. Colombia es un país de profundas rupturas en varios órdenes, de contrastes, y de enormes inequidades y tensiones, muchas de las cuales se expresan en la ruralidad. La deforestación y fragmentación de los bosques debido al aprovechamiento selectivo de especies maderables y la contaminación de ríos y fuentes de agua por minería y químicos derivados de la actividad agropecuaria, han perturbado los hábitats de muchas especies y roto el equilibrio ecológico, causando extinción de especies aun no descritas, y contribuyendo al aumento de gases de efecto invernadero y al cambio global. El impacto es de tal magnitud que esta destrucción y ruptura del equilibrio son consideradas causas concomitantes con la actual pandemia.

Esta emergencia ha dejado ver la interconexión de la economía y la ecología, develando, particularmente, la fragilidad de los sistemas agroalimentarios, pues su modelo de producción y consumo ha sobrepasado los límites ecológicos, generado un evidente deterioro de los recursos esenciales para la vida, como son el agua, el suelo, el aire, la agrobiodiversidad y los bosques. Adicionalmente Colombia se ha convertido en importador de alimentos básicos, destinando buena parte de sus tierras rurales a la producción de materias primas agroindustriales, con una disminución notable de las áreas de producción de alimentos de consumo directo. La globalización ha llevado a que los alimentos recorran grandes distancias, lo que hoy se dificulta por la crisis sanitaria, generando escasez y alto costo de los alimentos, con graves consecuencias para las vidas cotidianas de la mayoría de la población.

"Como Facultad que se ocupa, de los asuntos del Agro, asumimos los nuevos retos que plantea esta crisis para contribuir desde la investigacion, la docencia y la extensión a la restauración de los ecosistemas, así como a la reconstrucción y reactivación de sistemas agroalimentarios diversos y resilientes que garanticen el acceso a alimentos suficientes y saludables"

La pandemia COVID-19 confronta con nuestros estilos de vida; devela que la injusticia, la desigualdad y la extralimitación ecológica son cuestiones íntimamente relacionadas. Pero también nos brinda una gran oportunidad de repensarnos colectivamente, hacer los ajustes necesarios con el compromiso ético de inclusión, equidad y sostenibilidad. Por ello, como universidad pública y en particular como Facultad que se ocupa de los asuntos del Agro, asumimos los nuevos retos que plantea esta crisis para contribuir desde la investigación, la docencia y la extensión a la restauración de los ecosistemas, así como a la reconstrucción y reactivación de sistemas agroalimentarios diversos y resilientes que garanticen el acceso a alimentos suficientes y saludables, con posibilidades de un futuro para todos los seres vivos que generen beneficios sociales y ecológicos para las generaciones actuales y futuras.

En un nuevo entorno productivo se precisa diversificar para mejorar o encontrar modelos agrarios- agroindustriales que sean eficientes en el uso de la energía y de los recursos disponibles, económicamente viables, socialmente aceptados y técnicamente apropiados, que no degraden el medio ambiente. Se demanda, entonces, contribuir a la formulación de un modelo de desarrollo productivo en el marco de la competitividad y de la sostenibilidad. Lo anterior como evidencia de que la problemática de la producción ha evolucionado desde una dimensión, exclusivamente técnico-económica, a una dimensión social, cultural y ambiental.

Por todo lo anterior, el espacio rural debe ser dimensionado de manera que desborde el tradicional enfoque productivista de lo agroalimentario y forestal, y lo incluya como el espacio para recrear servicios ambientales, conservación y ordenamiento; y lo más importante, para hacer posible la justicia, el bienestar y legitimar la institucionalidad y la autoridad. La ruralidad, la agricultura y los espacios naturales son de interés para la sociedad en su conjunto y no sólo para la población que vive allí. La actual crisis obliga a recordar que el objetivo central de la educación es la formación de ciudadanos competentes en su trabajo y solidarios con sus congéneres. A esa tarea y desafío se compromete la Facultad de Ciencias Agrarias.

Facultad de Ciencias Agrarias
Universidad Nacional de Colombia
Sede Medellín

Research article

http://www.revistas.unal.edu.co/index.php/refame

Revista Facultad Nacional de Agronomía

Optimum harvest time for Kikuyu grass (*Cenchrus clandestinus*) according to the number of leaves per tiller and nitrogen fertilization

Momento óptimo de cosecha para el Kikuyo (*Cenchrus clandestinus*) de acuerdo al número de hojas por rebrote y la fertilización nitrogenada

doi: 10.15446/rfnam.v73n3.82257

Amanda Acero-Camelo[1]*, Martha L. Pabón[1], Gerhard Fischer[2] and Juan Evangelista Carulla-Fornaguera[1]

ABSTRACT

To determine the optimum harvest time of kikuyu grass (*Cenchrus clandestinus* (Hochst. ex Chiov.) Morrone), according to the number of leaves per tiller and nitrogen fertilization level, an experiment was carried out under greenhouse conditions using a 4×4 factorial design. The factors were the number of leaves per tiller (3, 4, 5, 6 leaves) and level of nitrogen (N) fertilization (0, 50, 100,150 kg of Nitrogen ha^{-1} $year^{-1}$). Dry matter (DM) yield and nutritional quality were determined. Additionally, the independent effect of nitrogen fertilization on undisturbed height, tiller density, and the phyllochron were evaluated. When the number of leaves per tiller increased (3 to 6), the leaf yield and dead forage also increased (P<0.05). The leaf-stem ratio (L:S) remained constant (P>0.05) among the number of leaves. Green forage-dead forage ratio (GF:DF) decreased (P<0.05) with a higher number of leaves per tiller. The concentration of crude protein (CP) decreased while the concentration of neutral detergent fiber (NDF) increased with an increment of the number of leaves per tiller (P<0.05). Nitrogen fertilization increased the undisturbed height, the density of tillers, DM yield from leaves, stems and green forage, GF:DF, and CP (P<0.05) while NDF decreased (P<0.05). The phyllochron was higher without N fertilization. The effects of the number of leaves per tiller and N fertilization on the most variables measured were independent of each other. However, due to an increment in DM yield caused by N fertilization, the pasture can be defoliated with fewer leaves per tiller, increasing defoliation frequency and improving forage quality.

Keywords:
Defoliation frequency
Leaf stage
Leaf to stem ratio
Pennisetum clandestinum

RESUMEN

Con el objetivo de determinar el momento óptimo de cosecha del kikuyo (*Cenchrus clandestinus* (Hochst. ex Chiov.) Morrone) de acuerdo al número de hojas por rebrote y la fertilización nitrogenada, se realizó un experimento bajo invernadero con un diseño factorial 4×4. Los factores fueron el número de hojas por rebrote a la cosecha (3, 4, 5, 6 hojas) y el nivel de fertilización nitrogenada (0, 50, 100, 150 kg de N ha^{-1} $año^{-1}$). Se determinó el rendimiento de materia seca (MS) y la calidad nutricional. Adicionalmente, se evaluó el efecto de la fertilización nitrogenada sobre la altura sin disturbar y el filocrono. Al aumentar el número de hojas por rebrote (3 a 6) aumentó (P<0,05) el rendimiento de hojas y el material muerto y disminuyó la relación material verde-muerto (P<0,05). La relación hoja-tallo fue constante entre número de hojas (P>0,05). Hubo menores concentraciones de proteína cruda (PC) y mayores concentraciones de fibra en detergente neutro (FDN) (P<0,05) a mayor número de hojas. La fertilización nitrogenada aumentó la altura sin disturbar, la densidad de estolones, la producción de MS, la relación forraje vivo-forraje muerto, la PC en hojas y disminuyó el FDN (P<0,05). El filocrono fue mayor (P<0,05) cuando no se fertilizó. Los efectos del número de hojas por rebrote y la fertilización nitrogenada fueron independientes para la mayoría de las variables. Sin embargo, debido al incremento en el rendimiento de MS por la fertilización N, la pastura puede ser defoliada con menor número de hojas por rebrote, aumentando la frecuencia de defoliación y mejorando la calidad del forraje.

Palabras clave:
Frecuencia de defoliación
Estado de hojas
Relación hoja tallo
Pennisetum clandestinum

[1] Facultad de Medicina Veterinaria y de Zootecnia. Universidad Nacional de Colombia. AA 7495, Bogotá, Colombia.
[2] Facultad de Ciencias Agrarias. Universidad Nacional de Colombia. AA 14490, Bogotá, Colombia.
* Corresponding author: <raaceroc@unal.edu.co>

Received: November 13, 2019; Accepted: March 24, 2020
Rev. Fac. Nac. Agron. Medellín 73(3): 9243-9253. 2020
ISSN 0304-2847 / e-ISSN 2248-7026

The optimum harvest time can be defined as a balance between the production of dry matter, nutritional quality, and grass persistence. As the age of the pasture increases, the production of dry matter increases, but the nutritional quality declines due to a reduction in the content of crude protein and digestibility and to the rise of neutral detergent fiber and acid detergent fiber (Canseco *et al*., 2007). As the plant begins senescence, the accumulation of dead forage occurs, and in some cases, leaf-stem ratio decreases, reducing the proportion of green forage allowance from the animal (Anwandter *et al*., 2007). This balance between quality and yield is achieved with adequate management of defoliation frequency, determined by the optimum harvest time. Additionally, defoliation frequency influences the number of reserves that the plant has for regrowth. When the pasture is defoliated early, in phenological age, it is possible that carbohydrate reserves and post grazing leaf area index are not enough to hold the subsequent regrowth, delaying growth and decreasing the annual production of dry matter, and sometimes, it can generate degradation of the pasture. On the other hand, if the pasture is defoliated too late, nutritional quality decreases, and the rate of accumulation of green forage is reduced (Chapman *et al*., 2011).

The optimum harvest time, where adequate dry matter production, nutritional quality, and pasture persistence can be achieved, cannot be assigned based on the chronological age. The plants grow at different rates depending on environmental conditions, in particular on the environmental temperatures (Andrade *et al*., 2015; Herrero *et al*., 2000). Therefore, plant characteristics have been identified (phenological state, flag leaf, among others) to make easy decision rules of the optimum harvest time to be used by the producer. One of these characteristics is the number of living leaves that remain in a stolon or a tiller, before the beginning of the senescence of the first leaf that emerged after grazing (Chapman *et al*., 2011). The number of green leaves that a tiller can support is constant and depends on the forage species (Fulkerson and Lowe, 2003). Leaf appearance rate is defined as the interval between the emergence of two leaves successive in the same tiller. Although this interval can be expressed in days, due to its close relation with environmental temperature, it has been calculated, preferably as thermal summation (phyllochron) (Wilhelm and McMaster, 1995). Knowing the phyllochron of a forage species allows making recommendations about defoliation frequency, considering the pasture phenological age and the mean temperature of a specific location.

Reeves and Fulkerson (1996) found for Kikuyu grass (*Cenchrus clandestinus* (Hochst. ex Chiov.) Morrone), the main forage species in high altitude tropics of Colombia (Mila and Corredor, 2004), that between 4 and 5 leaves per tiller, the grass is at the point of optimal nutritional quality and has enough carbohydrates reserves for the next regrowth. After this number of leaves, they found that the quality of Kikuyu began to decrease due to the senescence of older leaves. However, Fonseca *et al.* (2016) (in the Department of Boyacá, Colombia, at 2560 m.a.s.l.) concluded that the quality of Kikuyu did not change significantly between 4 and 6 leaves per tiller and the highest dry matter yield was reached in the stage of 6 leaves.

Herrero *et al*. (2000), using a growth model for this species, suggested that the optimal number of leaves per tiller to harvest could be influenced by the level of nitrogen fertilization. Without nitrogen fertilization, the model estimated 7 as the optimum number of leaves per tiller to harvest Kikuyu. At fewer leaves per tiller the forage mass required for grazing (>2500 kg dry matter ha^{-1}) would not be reached. With N fertilization (100 kg N ha^{-1}), the number of the leaves needed to have enough dry matter yield for grazing would be lower (4 to 6). These authors suggested that N fertilization increases the rate of leaf elongation and, at the same time, the rate of forage mass accumulation, reducing the time required to accumulate enough dry matter yield for grazing. A positive response in the growth rate induced by N fertilization has been proven in other studies with forage grasses (Borrajo and Alonso, 2014; Almeida *et al*., 2011). This response is more related to the effect of nitrogen on the production of cells rather than its elongation, although this mechanism is still under discussion (Gastal *et al*., 2015).

According to the above mentioned, the objective of this study was to determine the optimal harvest time of Kikuyu grass using as criteria the number of living leaves per tiller and to establish if the N fertilization affects this number.

MATERIALS AND METHODS

The experiment was carried out from December 2015 to June 2016 at the Facultad de Ciencias Agrarias of the Universidad Nacional de Colombia, Bogotá, at 2600 m.a.s.l. and an average daily temperature of 14 °C.

The experiment was conducted in pots in a greenhouse, with a permanent record of temperature and relative humidity (Table 1).

Kikuyu grass stolons were planted in 96 plastic pots (34 cm×27 cm×12 cm), with a density of 12 stolons per pot, using the same kind of soil (from high altitudes >2,800 m.a.s.l.) for all pots. This soil presented dark coloration, high content of organic matter (13.8%), strong acidity (pH 5.4), suitable Cation-Exchange Capacity (54.8 meq per 100 g), and clay loam texture, aspects similar to those reported for soils from the bleak Colombian uplands (IDEAM, 1999). Irrigation was applied to maintain soil moisture at field capacity. Kikuyu stolons were allowed to grow until they covered at least 80% of the pot. This period lasted 4 months. At this time, uniform defoliation was made at 3 cm from the ground.

Table 1. Mean temperature and relative humidity during experimental periods.

Month	Mean temperature (°C)	Relative humidity (%)
April	19.5	73.7
May	18.5	75.4
June	17.5	72.3

Treatments and variables

Sixteen treatments were evaluated in a 4×4 factorial design, with three repetitions per treatment; each repetition consisted of 2 pots. Leaves per tiller at the time of harvest and the level of nitrogen fertilization were the factors. Four harvest treatments considering the leaves per tiller 3 (L3), 4 (L4), 5 (L5) and 6 (L6), and four levels of nitrogen fertilization, 0 (N0), 50 (N50), 100 (N100) and 150 (N150) kg N ha^{-1} $year^{-1}$ were studied. The nitrogen source used was urea. Additionally, applications of phosphorus (69 kg ha^{-1} $year^{-1}$), potassium (90 kg ha^{-1} $year^{-1}$), and magnesium sulfate (25 kg ha^{-1} $year^{-1}$) were made, according to the requirements of the Kikuyu (Bernal and Espinosa, 2003) in all treatments.

Dry matter (DM) yield (extrapolated to t ha^{-1}) of leaves, stems, green forage (GF, leaves+stems), dead forage (DF), leaf-stem ratio (L:S), and green forage-dead forage ratios (GF:DF) were the variables evaluated. Also, the content of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, ether extract (EE) and ash were determined for leaves and stems.

Additionally, eight pots were taken at random of each level of nitrogen to measure undisturbed height, tiller density, and leaf appearance rate and to evaluate the effect of N fertilization on these variables.

Sample measurements and analysis

After the uniform defoliation, the measurements of leaf appearance, undisturbed height, and tiller density were initiated. Undisturbed height was measured at ground level in five points of each pot, and the total tillers of each pot were counted. A tiller from each pot was marked, and measurements for leaf appearance rate were made each four days until the pot was harvest. The leaf appearance rate was determined, expressed as the degree days necessary for the appearance of a leaf (phyllochron) using equation 1.

$$TT = \sum_{i=1}^{n} T_i - nT_b \qquad (1)$$

Where:
TT: Thermal time for the appearance of a leaf,
T_i: Mean daily temperature (°C) per day *i*,
T_b: Base temperature (°C) –this parameter was set at 8 °C, according to Ivory and Whiteman (1978).

Weekly, five tillers per pot were taken at random to count the number of leaves completely expanded by stolon.

When the average of all pots within a treatment reached the number of leaves corresponding to their treatment, they were harvested, and the leaf, stem, and DF fractions were separated to perform the corresponding analyzes. Samples of the leaf, stem and dead material fractions were dried at 65 °C for 48 hours and then ground. Contents of CP, NDF, ADF, EE, ash, and lignin were estimated by Near-infrared spectroscopy (NIRS), according to the equations generated for Kikuyu grass by Ariza-Nieto *et al*. (2018).

Statistical analysis

Three different experimental models were used. A completely randomized model with factorial arrangement 4×4 (leaves per tiller × nitrogen fertilization) (Martínez *et al*., 2011) was used for the DM production from leaves, stems, GF:DF, stem-leaf ratio and for the nutritional composition of leaves and stems. A repeated measurement design (Martínez *et al*., 2011) was used for the analysis of the variable undisturbed height to determine the effect of nitrogen fertilization on the density of tillers and phyllochron. Homogeneity of the experimental material and normal distribution of the experimental error, with mean zero and common variance, were examined. Some variables were transformed to comply with the homogeneity of variances. The lack of normality was accepted when the data achieved the assumption of homogeneity of variances with the Levene test, which is considered robust to the lack of normality. The comparison of means for all the variables was made by the Tukey test. Hotelling test was used for the undisturbed height. The data were analyzed with InfoStat software, 2016l version.

RESULTS AND DISCUSSION

Dry matter yield

The interaction between numbers of leaves per tiller and nitrogen fertilization was not significant for DM yield of leaves per tiller (P=0.0751), stems (P=0.2616), GF (P=0.0790), L:S (P=0.7331), and GF:DF (P=0.8976). The interaction was only significant for DF (P=0.0451). DM yield of leaves and green forage increased when Kikuyu pasture was harvested with more leaves per tiller and/or fertilized with nitrogen. However, differences among harvest time (leaves per tiller) were small and only evident between L3 and L6. N fertilization differences were more evident at 50 kg N ha^{-1} year^{-1} (Figures 1 and 2). The highest leaf yield was reached at 150 kg N ha^{-1} year^{-1} (Figure 2). Stem yield increased with nitrogen fertilization, but the effect of leaves per tiller was not evident (Figures 1 and 2). Dead forage increased when the Kikuyu was harvested with more than 5 leaves per tiller. The magnitude of the response varied due to N fertilization (Interaction, P<0.0451), where differences among the leaves per tiller treatment were not evident for the treatment without N (Figure 3).

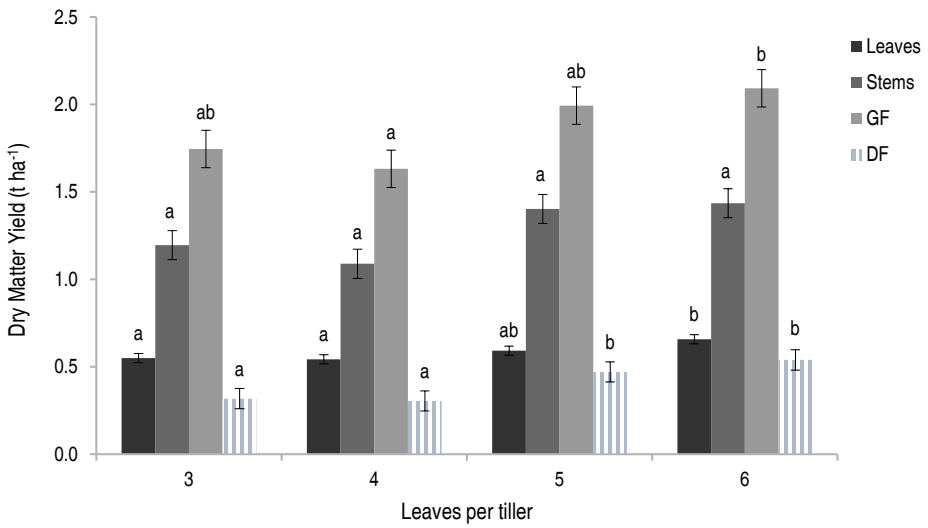


Figure 1. Dry Matter yield of leaves, stems, green forage (GF), and dead forage (DF) of kikuyo defoliated at a different number of leaves per tiller. Different letters within forage component (leaf, stem, GF, DF) mean significant difference (P<0.05).

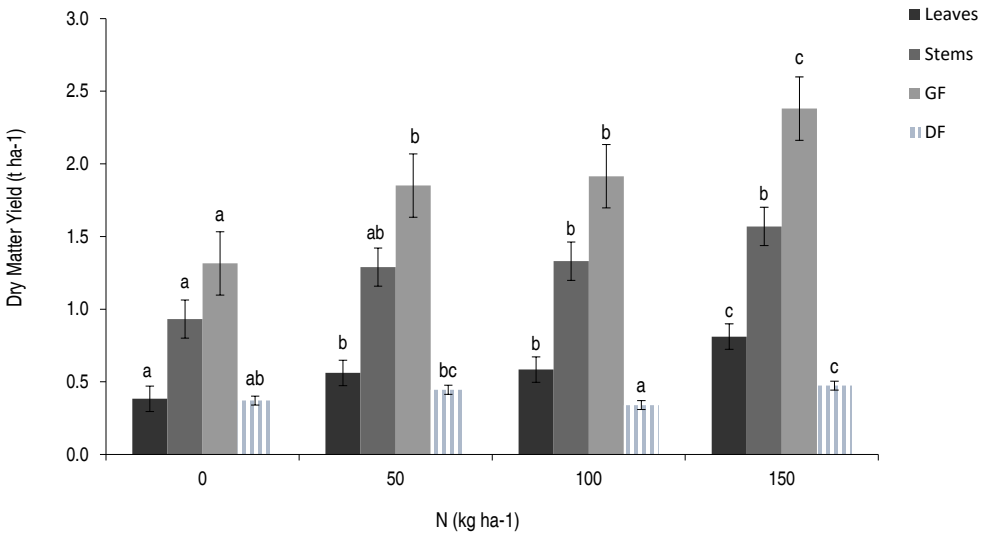


Figure 2. Dry Matter yield of leaves, stems, green forage (GF), and dead forage (DF) of kikuyu fertilized with differen doses of nitrogen. Different letters within forage component (leaf, stem, GF, DF) mean significant difference (P<0.05).

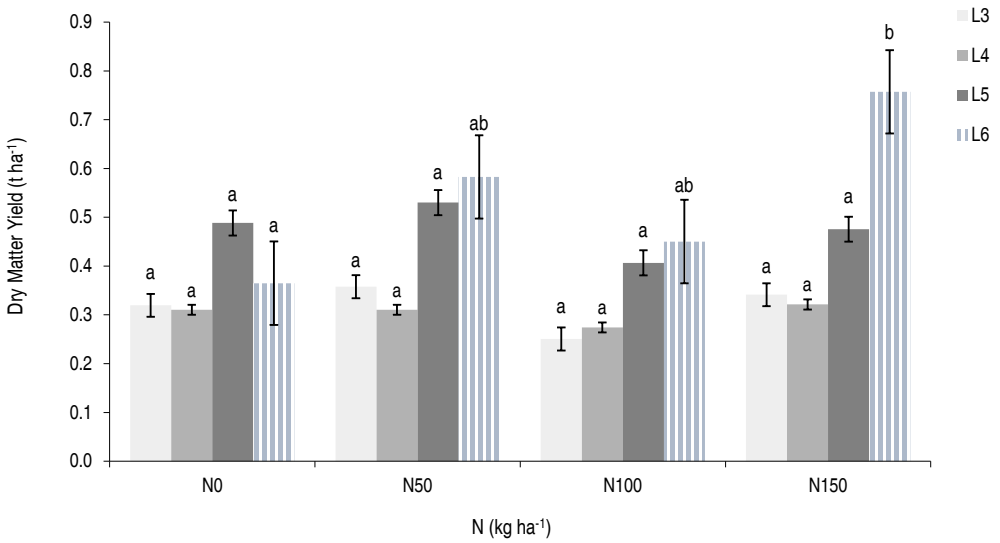


Figure 3. Yield of dead forage (DF) in kikuyu according to nitrogen fertilization and defoliation at a different number of leaves per tiller. Different letters within the number of leaves per tiller mean significant difference (P<0.05).

Previous researches recommended harvesting Kikuyu at a maximum leaf stage of 4.5 since the L:S begins to decrease after 4 leaves per tiller (Fulkerson *et al*., 1999; Reeves and Fulkerson, 1996). In this experiment, the L:S remained constant regardless of the number of leaves per tiller, and it was not influenced by nitrogen fertilization (Figures 4 and 5). This result is similar to Fonseca's *et al*. (2016) study, which suggests that the changes in the proportion of stems in Kikuyu grasslands for the 4 to 6 leaf stages do not occur under the high altitude conditions in the tropics, maintaining a correlation between the number of leaves and stems within the pasture, given its stoloniferous growth habit. Fulkerson *et al*. (1999) found that the proportion of stems increased among leaf stages, with proportions of 9.5%, 13.1%, and 20% for 2, 4, and 6 leaves, respectively. However, this response varies by season. For summer, there were no differences

in the proportion of stems among pasture harvested at different numbers of leaves per tiller. In autumn-winter, when the weather conditions are similar to those of the high altitude tropics of Colombia, L:S was similar between pasture harvested at 4 and 6 leaves per tiller and only differed to the grass harvested at 2 leaves per tiller.

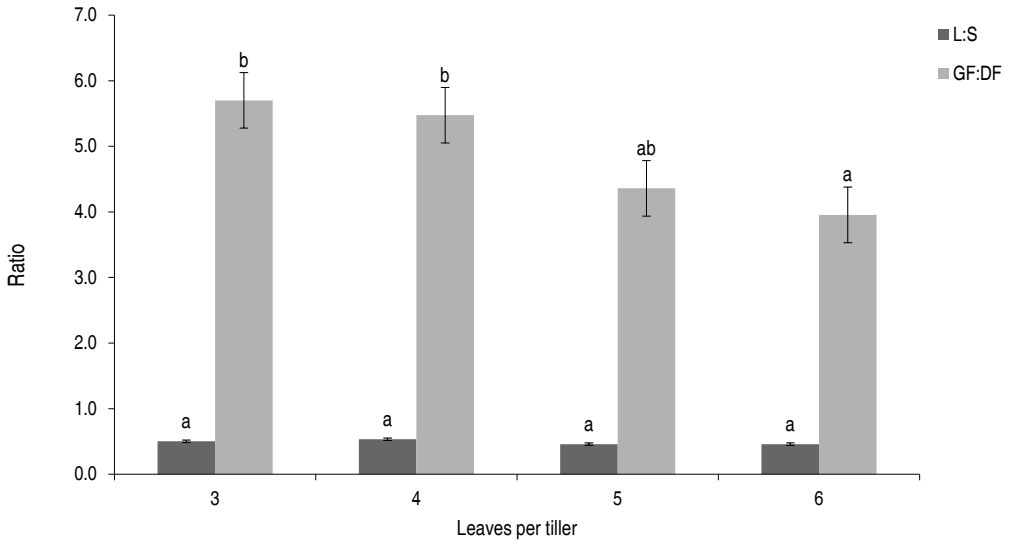


Figure 4. Leaf-stem ratio (L:S) and green forage-dead forage ratio (GF:DF) according to the kikuyu defoliation at a different number of leaves per tiller. Different letters within a ratio mean significant difference (P<0.05).

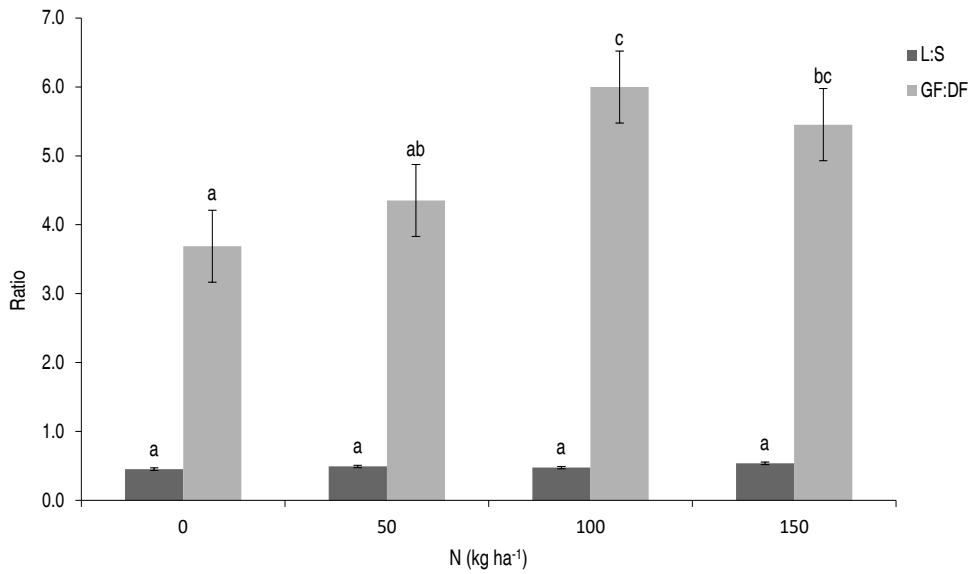


Figure 5. Effect of the nitrogen fertilization on leaf: stem ratio (L:S) and green forage-dead forage ratio (GF:DF). Different letters within a ratio mean significant difference (P<0.05).

In the present study, L:S was close to 0.5, similar to previous studies (Ivory and Whiteman, 1978), in which the whole plant was harvested, including the stolons at ground level, which may have generated a greater production of DM from stems than from leaves. The GF:DF was reduced when Kikuyu was harvested with more leaves per tiller (Figure 4) but increased by N fertilization (Figure 5). Changes in this ratio were insignificant between leaves 3 and 4 in a tiller that had similar ratios, but the changes were accelerated after 4 leaves (Figure 4). These results

are comparable to the study reported by Reeves and Fulkerson (1996), who found the proportion of DF increased substantially after 4 leaves per tiller.

Different authors have reported the positive response of Kikuyu to nitrogen fertilization regarding yield and quality (García *et al*., 2014; Mejía-Taborda *et al*., 2014; Dugmore, 2011; Castillo *et al*., 1983). In this research, N fertilization resulted in a higher yield of DM of leaves, stems, and GF, reaching the highest production with N150 (Figure 2) compared to analogous studies. Although nitrogen fertilization also increased the yield of dead forage, N improved the GF:DF ratio, suggesting a positive net effect of N on pasture quality. The best results were obtained with N100 and N150 (Figure 5). Castillo *et al.* (1983) found a favorable response in the growth rate of Kikuyu with levels of up to 500 kg N ha^{-1} $year^{-1}$, but with a lower economic efficiency because of the increase in the quantity of N applied. So the optimal level of application is a function of profitability, and it is not related to the maximum response of the species. Herrero *et al*. (2000) suggested the best harvest time (leaves per tiller) for Kikuyu will depend on N fertilization. In the present study, the effects on yield and chemical composition of harvesting Kikuyu at different leaves per tiller were independent of the effects of N fertilization. Moreover, when Kikuyu was not fertilized or even with low levels of fertilization (N50), it was not possible to reach the recommended biomass to grazing (2,600 kg DM ha^{-1}) (García *et al*., 2014). Therefore, the leaf stage is not a unique criterion for harvesting time. It should be considered the undisturbed height and pre-grazing cover to define the optimum grazing time. Finally, N fertilization could reduce pasture rest period (lower leaf stages), to reach higher annual forage yields of better quality.

Chemical composition

The interaction between numbers of leaves per tiller and nitrogen fertilization was not significant for CP (*P*=0.4052), NDF (*P*=0.9148), ADF (*P* =0.9462), lignin (*P*=0.6827), EE (*P*=0.4868) and ashes (*P*=0.1000).

By increasing the number of leaves per tiller at harvest, the level of crude protein in both leaves and stems was slightly reduced. This difference was only significant between the Kikuyu harvested at L3 and L6 (Table 2). NDF content in leaves was higher as tillers had more leaves, but not on stems (Table 2). In the leaves, the ADF and the ash were not modified regarding the increase in the number of leaves per tiller at harvest. However, in the stems, the contents of ADF and ash were reduced, being lower at L6 (Table 2).

Table 2. Chemical composition of Kikuyu leaves and stems defoliated at a different number of leaves per tiller.

Variable	L3	L4	L5	L6	SE	*P*
			%DM			
			Leaves			
CP	10.7 b	10.1 ab	9.6 a	9.6 a	0.19	0.0004
NDF	62.6 a	63.3 ab	64.0 b	63.5 ab	0.23	0.0023
ADF	30.2	29.9	30.0	30.2	0.22	0.7015
Lignin	6.8	6.3	6.6	6.5	0.14	0.0740
EE	1.8	1.7	1.7	1.8	0.02	0.0151
Ashes	9.8	9.6	9.8	9.5	0.15	0.3833
			Stems			
CP	3.8 b	3.8 b	3.4 ab	3.0 a	0.19	0.0018
NDF	62.6 b	61.4 ab	58.7 a	58.6 a	0.83	0.0034
ADF	28.4 a	28.0 a	26.4 a	26.5 a	0.55	0.0305
Lignin	7.0 b	6.3 ab	5.6 a	5.6 a	0.25	0.0011
EE	1.26	1.30	1.31	1.37	0.04	0.2433
Ashes	4.9 c	5.0 c	4.3 b	3.6 a	0.12	<0.0001

SE: standard error of the mean.
Different letters within a row mean significant difference (*P*<0.05).

Quality of kikuyo is already changing at L5, reducing the content of CP and increasing the content of NDF with respect to L3. However, no significant differences were found between L4, L5, and L6, similar to what was found by Fonseca *et al.* (2016) and contrary to the study reported by Reeves and Fulkerson (1996). The content of NDF and lignin of L5 and L6 respect to L3 and L4 were inferior. This could be explained by the appearance of a new secondary tiller in L5 and L6, which have lower cell wall content, while in L3 and L4, primary tillers were dominant.

The results of this experiment show that the best quality of the Kikuyu is found in L3. Yet, at this stage, it may be challenging to reach enough biomass for grazing. So to harvest the pasture may be recommended between L4 and L5 stages. Although in the L6 stage, there were no significant differences in nutritional quality with respect to L4 and L5, senescence losses began in the pasture, with a negative impact on the efficiency of use. Escobar *et al.* (2020) reported the highest nutrient yields (CP, Net Energy for Lactation [NEL]) for L5 in a ten-month on-farm trial without N fertilization. Harvesting Kikuyu with fewer leaves per tiller (<5) may be feasible if nitrogen fertilization is applied or cultivated in high fertile soils (García *et al.*, 2014; Dugmore, 2011; Mejía-Taborda *et al.*, 2014; Castillo *et al.*, 1983).

Nitrogen fertilization had a positive impact on the chemical composition of Kikuyu. Similar results were found by Dugmore (2011) and Castillo *et al.* (1983). The content of CP in leaves increased by N fertilization, while the content of NDF in the leaves and stems decreased (Table 3). The high content of NDF in Kikuyu is considered limiting in terms of nutritional quality, which can restrict its intake. (García *et al.*, 2014; Correa *et al.*, 2008). The reduction in NDF by nitrogen fertilization was reached with levels from 0 to 500 kg ha^{-1} $year^{-1}$, regardless of the source of nitrogen used, achieving 53.5% as the minimum content of NDF (Castillo *et al.*, 1983). Therefore, it is possible to reduce the NDF content by nitrogen fertilization, within the ranges of the species and counteract the negative effect on DM intake.

Table 3. Chemical composition of Kikuyu leaves and stems with different levels of nitrogen fertilization.

Variable	N0	N50	N100	N150	SE	*P*
	%DM					
Leaves						
CP	9.0 a	9.7 ab	10.1 b	11.4 c	0.19	<0.0001
NDF	64.4 c	63.6 cb	63.2 b	62.2 a	0.23	<0.0001
ADF	30.4	30.2	29.9	29.9	0.22	0.4176
Lignin	6.9 b	6.5 ab	6.3 a	6.5 ab	0.14	0.0319
EE	1.7	1.8	1.8	1.8	0.02	0.1437
Ashes	9.8	9.6	9.6	9.8	0.15	0.4335
Stems						
CP	3.3	3.4	3.5	3.8	0.16	0.2127
NDF	62.4 b	59.0 a	60.2 ab	59.7 ab	0.88	0.0485
ADF	28.6	26.8	27.3	26.7	0.58	0.1171
Lignin	6.7	5.7	6.0	6.1	0.27	0.0661
EE	1.28	1.31	1.38	1.26	0.04	0.1572
Ashes	4.3 a	4.2 a	4.4 a	4.9 b	0.12	0.0030

SE: standard error of the mean.
Different letters within a row mean significant difference (P<0.05).

The values obtained for CP and NDF differ significantly from those found in field conditions for Kikuyu. In general, CP values were lower, and NDF values were higher than those (17.8±0.9% and 58.3±1.5%, respectively) reported by García *et al.* (2014). It could be due to the characteristics of the soil used, which was highly restrictive in the supply of nitrogen (Yutaro and Osamu, 2018). The high organic matter content in soils from bleak

uplands has been related to low mineralization due to the low temperatures present at high altitudes that limit biological activity (IDEAM, 1999). These characteristics of the soil led to the N0 treatment being very limiting in nitrogen for the plant, an aspect reflected in the poor growth obtained and in difficulty for the establishment of the Kikuyu in the pots of this treatment.

Structural variables and phyllochron

The undisturbed pasture height and density of Kikuyu tillers were increased by N fertilization, while the phyllochron was reduced. The effects of N on tiller density was only evident for the highest level of N fertilization (Table 4). The increase of N fertilization has been related to a higher foliar elongation (Borrajo and Alonso, 2014; Da Silva *et al*., 2012; Almeida *et al*., 2011). Therefore, nitrogen deficiency may be related to the slow elongation of Kikuyu pastures observed in low fertility soil used in this experiment, and greater height of the pasture in response to N. Previous studies also had reported N fertilization has a positive effect on tilling (Dourado *et al*., 2015, Da Silva *et al*., 2012). This effect may be greater in plants that grow distant from each other than in dense stoloniferous growth species such as Kikuyu. These pastures are usually denser, and the light competition may limit the increments in tiller density (Lemaire *et al*., 2009).

Table 4. Effect of nitrogen fertilization on undisturbed height, tiller density and phyllochron.

Trait	N0	N50	N100	N150	SE	*P*
Undisturbed height (cm)	19.2 a	21.9 b	23.7 c	23.9 c	0.23	<0.0001
Tiller density (# per pot)	99.7 a	112.5 a	109.4 a	132.9 b	4.70	0.0001
Phyllochron (GDD)	150.4 a	121.0 b	136.4 ab	115.5 b	9.15	0.0380

GDD: Growing Degree Days for the appearance of a leaf; SE: standard error of the mean.
Different letters within a row mean significant difference (P<0.05).

The leaf appearance rate is primarily influenced by temperature and then by soil moisture and nutrient content (Wilhelm and McMaster, 1995). It was observed in this experiment, without N fertilization (N0) since Kikuyu had a higher phyllochron while in the other N fertilization levels were similar (Table 4). Wilhelm and McMaster (1995) concluded that under extreme conditions of soil fertility, like N0 treatment, the phyllochron may be increased due to a longer time required for the appearance of a leaf. Therefore, it limits the accumulation of DM.

CONCLUSIONS

Harvesting Kikuyu with higher leaves per tiller (>5) or fertilized with N increased DM yield. However, the best nutritional quality was obtained harvesting with fewer leaves (<4), and fertilizing with higher concentrations of N. DM yield was 1.7 t ha^{-1} harvesting at a lower number of leaves per tiller, which is not suitable to grazing regardless of the positive effect of N fertilization. It could be recommended to harvest the Kikuyu between 4 and 5 leaves per tiller to maintain the balance between quantity and quality. Harvesting Kikuyu, with fewer leaves per tiller than 4, would be feasible if pastures are fertilized with higher levels of N (>100 kg ha^{-1}). Regardless of the number of leaves per tiller, fertilization can maximize DM production and contributes to the improvement of nutritional quality by increasing the contents of CP and decreasing NDF contents in leaves and stems. Likewise, it improves GF:DF, the production of DM such as leaves, and the undisturbed height of the pasture. Therefore, N fertilization is a key element to improve DM yield, the nutritional quality of the pasture, and to increase defoliation frequency. Additionally, this study suggests that the number of leaves per tiller cannot be used as the only criteria to define the moment to graze. Also, pre-grazing biomass available and the undisturbed height of the pasture should be considered.

ACKNOWLEDGMENTS

The authors thank the Gobernación de Cundinamarca and the Sistema General de Regalías (SGR) which founded this research through the project “Innovación, ciencia y tecnología para productores de leche en la provincial de Ubaté”. The authors thank the Ministerio de Ciencia, Tecnología e Innovación de Colombia which support the graduate student fellowship.

REFERENCES

Almeida ACS, Mingoti R, Coelho R, and Lourenço LF. 2011. Simulação do crescimento do capim Tanzânia irrigado com base na unidade fototérmica, na adubação nitrogenada e na disponibilidade hídrica do período. Acta Scientiarum. Agronomy 33(2): 215-222. doi: 10.4025/actasciagron.v33i2.4901

Andrade AS, Santos PM, Pezzopane JRM, de Araujo LC, Pedreira BC, Pedreira CGS, Marin FR, and Lara MAS. 2015. Simulating tropical forage growth and biomass accumulation: an overview of model development and application. Grass and Forage Science 71:54-65. doi:10.1111/gfs.12177

Anwandter V, Balocchi O, Parga J, Canseco C, Teuber N, Abarzúa A, Lopetegui J y Demanet R. 2007. Capítulo 6: Métodos y control del pastoreo, pp. 91-105. En: Teuber N, Balocchi O and Parga J. (eds.). Manejo del Pastoreo. Imprenta América. Osorno, Chile.

Ariza-Nieto C, Mayorga OL, Mojica B, Parra D and Afanador-Tellez G. 2018. Use of LOCAL algorithm with near infrared spectroscopy in forage resources for grazing systems in Colombia. Journal of Near Infrared Spectroscopy 26(1):44–52. doi: 10.1177/0967033517746900

Bernal J y Espinosa J. 2003. Manual de nutrición y fertilización de pastos. pp. 94. International Plant Nutrition Institute. Quito, Ecuador.

Borrajo I y Alonso S. 2014. Tasa de elongación foliar en materiales de agropiro alargado: efecto de la fenología y el agregado de nitrógeno. En: 37° Congreso AAPA – 2nd Joint Meeting ASAS-AAPA – XXXIX Congreso de la Sociedad Chilena de Producción Animal. Octubre 20, 21 y 22 de 2014.

Canseco C, Abarzúa A, Parga J, Teuber N y Balocchi O. 2007. Capítulo 4: Calidad nutritiva de las praderas, pp. 51-67. En: Teuber N, Balocchi O and Parga J. (eds.). Manejo del Pastoreo. Imprenta América. Osorno, Chile.

Castillo E, Coward J, Sánchez JM, Jiménez C y López C. 1983. Efecto de la fertilización nitrogenada en la época lluviosa sobre productividad, composición química y digestibilidad *in vitro* del pasto kikuyo bajo pastoreo en el Cantón de Coronado. Revista Agronomía Costarricense 7 (1/2): 9-15.

Correa HJ, Carulla JE y Pabón ML. 2008. Valor nutricional del pasto kikuyo (*Pennisetum clandestinum* Hoechst Ex Chiov.) para la producción de leche en Colombia (Una revisión): I - Composición química y digestibilidad ruminal y posruminal. Livestock Research for Rural Development 20(4).

Chapman DF, Tharmaraj J, Agnusdei M and Hill J. 2011. Regrowth dynamics and grazing decision rules: further analysis for dairy production systems based on perennial ryegrass (*Lolium perenne*) pastures. Grass and Forage Science 67(1):77-95. doi: 10.1111/j.1365-2494.2011.00824.x

Da Silva TC, Perazzo AF, Macedo CHO, Batista ED, Pinho RMA, Bezerra HFC, Santos E. 2012. Morfogênese e estrutura de *Brachiaria decumbens* em resposta ao corte e adubação nitrogenada. Archivos de Zootecnia 61(233): 91-102. doi: 10.4321/S0004-05922012000100010

Dourado RL, Souza AL, Zanine AM, Toral FLB, Ferreira DJ and Abreu JG. 2015. Structural and production characteristics of Piatã grass forage submitted to levels of nitrogen. American Journal of Plant Sciences 6(5): 693-701. doi: 10.4236/ajps.2015.65075

Dugmore T. 2011. The effect of nitrogen fertilization and stage of regrowth on the nutritive value of kikuyu in the midlands of KwaZulu-Natal (PhD. Thesis). School of Agricultural Sciences and Agribusines, University of KwaZulu-Natal. Pietermaritzburg, Southafrica. pp. 20-34.

Escobar CM, Cardenas RE, Carulla FJ. 2020. Efecto de la altitud y la frecuencia de defoliación en la calidad y crecimiento del pasto Kikuyo (*Cenchrus clandestinus*). Revista Facultad Nacional de Agronomía Medellín 73(1): 9121-9130. doi: 10.15446/rfnam.v73n1.77330

Fonseca C, Balocchi O, Keim JP y Rodríguez C. 2016. Efecto de la frecuencia de defoliación en el rendimiento y composición nutricional de *Pennisetum clandestinum* Hochst. ex Chiov. Agrosur 44(3): 67-76. doi: 10.4206/agrosur.2016.v44n3-07

Fulkerson WJ, Slack K and Havilah E. 1999. The effect of defoliation interval and height on growth and herbage quality of kikuyu grass (*Pennisetum clandestinum*). Tropical Grasslands 33(3): 138-145.

Fulkerson WJ and Lowe KF. 2003. Grazing management. pp. 1142–1149. In: Roginski H, Fuquay JW and Fox PF (eds.). Encyclopedia of dairy science (Vol. 2). Academic Press, Waltham, MA, USA.

García SC, Islam MR, Clark CEF and Martin PM. 2014. Kikuyu-based pasture for dairy production: a review. Crop and Pasture Science. Journal Compilation CSIRO. doi: 10.1071/CP13414

Gastal F, Lemaire G, Durand JL and Louarn G. 2015. Chapter 8. Quantifying crop responses to nitrogen and avenues to improve nitrogen-use efficiency, pp.161-206. In: Sadras V, Calderini D (eds.). Crop Physiology: Applications for Genetic Improvement and Agronomy. Elsevier Inc.

Herrero M, Fawcett RH and Dent JB. 2000. Modelling the growth and utilisation of kikuyu grass (*Pennisetum clandestinum*) under grazing. 2. Model validation and analysis of management practices. Agricultural Systems 65(2): 99-111. doi: 10.1016/S0308-521X(00)00028-7

IDEAM. Instituto de Hidrología, Meteorología y Estudios Ambientales. 1999. El Macizo Colombiano y su Área de Influencia. Bogotá, Colombia. pp. 83-84.

Ivory DA and Whiteman PC. 1978. Effect of temperature on growth of five subtropical grasses. II. Effect of low night temperature. Australian Journal of Plant Physiology 5(2): 149-157. doi: 10.1071/PP9780149

Lemaire G, Da Silva SC, Agnusdei M, Wade M and Hodgson J. 2009. Interactions between leaf lifespan and defoliation frequency in temperate and tropical pastures: a review. Grass and Forage Science 64(4): 341-353. doi: 10.1111/j.1365-2494.2009.00707.x

Martínez BR, Martínez RN y Martínez M. 2011. Diseño de experimentos en ciencias agropecuarias y biológicas con SAS, SPSS, R y STATISTIX. Tomo I. Fondo Nacional Universitario. Bogotá, Colombia.

Mejía-Taborda AC, Ochoa-Ochoa R and Medina-Sierra M. 2014. Effect of different doses of compound fertilizer on the quality of kikuyu grass (*Pennisetum cladestinum* Hochst. Ex Chiov.). Pastos Forrajes 37(1):31-37.

Mila A y Corredor G. 2004. Evaluación de la composición botánica de una pradera de Kikuyo (*Pennisetum clandestinum*) recuperada mediante escarificación mecánica y fertilización con compost. Revista Corpoica 5(1): 70-75. doi: 10.21930/rcta.vol5_num1_art:28

Reeves M and Fulkerson WJ. 1996. Establishment of an optimal grazing time of kikuyu pastures for dairy cows. p. 4. In: Proceedings of the 8th Australian Agronomy Conference; 1996

January 30 – February 2, Toowoomba, Queensland.

Wilhelm W and McMaster GS. 1995. Phyllochron in grass development and growth In: Symposium on the Phyllochron. Crop Science 35(1): 1-3.

Yutaro M and Osamu U. 2018. Structural and physiological responses of the C4 grass *Sorghum bicolor* to nitrogen limitation. Plant Production Science 21(1):39-50. doi:10.1080/1343943X.2018.1432290

Research article

http://www.revistas.unal.edu.co/index.php/refame

Revista Facultad Nacional de Agronomía

UNIVERSIDAD NACIONAL DE COLOMBIA

Effect of nitrogen and phosphorus fertilization sources on the potato crop yield (*Solanum tuberosum* L.)

Efecto de fuentes de fertilización nitrogenada y fosforada en el rendimiento del cultivo de papa (*Solanum tuberosum* L.)

doi: 10.15446/rfnam.v73n3.82624

Rember Pinedo-Taco[1]*, Tulio Olivas-Alvarado[2], Gilberto Rodríguez-Soto[1] and Viviana Castro-Cepero[3]

Keywords:
Alkaline soils
Essential plant nutrient
Fertilizers
Potato yield
Solanum tuberosum

ABSTRACT

Potato (*Solanum tuberosum* L.) is a demanding crop regarding fertilization practices, and its productivity also depends on the variety used, environmental conditions, soil fertility, and crop management. The aim of this study was to investigate the effect of the interaction of nitrogen and phosphorus fertilization sources on potato crop yield. A randomized block design with a 3×3 factorial arrangement, nine treatments, and four repetitions was established. INIA-303 Canchan was the variety evaluated using three nitrogen fertilization sources: ammonium nitrate (AN), ammonium sulfate (AS), and urea. Also, three phosphorus sources were used: monoammonium phosphate (MAP), diammonium phosphate (DAP), and triple superphosphate (TSP). Total yield, commercial yield, tuber number per plant, and tuber weight per plant were studied. INIA-303 Canchan variety showed positive responses to the combinations of nitrogen and phosphorus fertilization according to the soil and climate conditions where the research was carried out. AN+DAP was the combination with the highest total yield, commercial yield, number, and weight of tubers per plant (P<0.01).The fertilization mixtures of AN+DAP, AS+MAP, and AN+MAP, applied on the INIA-303 Canchan potato variety, can be recommended to achieve yields between 32.45 t ha^{-1} and 33.98 t ha^{-1}.

Palabras clave:
Suelos alcalinos
Nutrientes vegetales esenciales
Fertilizantes
Rendimiento de papa
Solanum tuberosum

RESUMEN

La papa (*Solanum tuberosum* L.) es un cultivo exigente con respecto a las prácticas de fertilización, y su productividad también depende de la variedad utilizada, las condiciones ambientales, la fertilidad del suelo y el manejo del cultivo. El objetivo de este estudio fue investigar el efecto de interacción de las fuentes de fertilización nitrogenadas y fosforadas en el rendimiento del cultivo de papa. Se estableció un diseño factorial 3×3 de bloques al azar con, nueve tratamientos y cuatro repeticiones. La variedad evaluada fue INIA-303 Canchan con tres fuentes de fertilización nitrogenada: nitrato de amonio (NA), sulfato de amonio (SA) y urea, y tres fuentes de fósforo: fosfato monoamónico (FMA), fosfato diamónico (FDM) y superfosfato triple (SFT). Las variables estudiadas fueron rendimiento total, rendimiento comercial, número de tubérculos por planta, peso del tubérculo por planta. La variedad INIA-303 Canchan, mostró respuestas positivas a las combinaciones de fuentes de fertilización de nitrógeno y fósforo bajo las condiciones del suelo y el clima donde se realizó la investigación. En la combinación NA+FDM se encontró el mayor rendimiento total, rendimiento comercial, número y peso de tubérculos por planta (P<0.01). Las mezclas recomendadas para la fertilización de la variedad de papa Canchan INIA-303, para lograr rendimientos entre 32,45 t ha^{-1} a 33,98 t ha^{-1}, bajo las condiciones del área de estudio son NA+FDM, SA+FMA y NA+FMA.

[1] Departamento de Fitotecnia. Facultad de Agronomía. Universidad Nacional Agraria La Molina. Av. La Molina. AP 12-056, Lima, Perú.
[2] Drokasa Perú S.A. Av. Calca 142, Cercado de Lima. CP 15012, Lima, Perú.
[3] Departamento de Biología. Facultad de Ciencias. Universidad Nacional Agraria La Molina. Avenida La Molina. AP 12-056, Lima, Perú.
* Corresponding author: <rpinedo@lamolina.edu.pe>

Received: February 4, 2020; Accepted: May 7, 2020
Rev. Fac. Nac. Agron. Medellín 73(3): 9255-9261. 2020

ISSN 0304-2847 / e-ISSN 2248-7026

Potato (*Solanum tuberosum* L.) is one of the most important crops in Peru in economic and food terms. The high Andean region has the biggest surface of the crop, with more than 730,000 producers in 317,647 ha approximately. However, at the coastal level, it is managed with improved varieties (Pradel *et al*., 2017). The INIA-303 Canchan INIA variety was produced by the National Institute of Agricultural Innovation (INIA), showing precocity, resistance to *Phytophthora*, and capability of adapting to various agroclimatic conditions (Fonseca, 1996). It is considered one of the most used varieties in potato production in the Andean region and the Peruvian coast (Egusquiza, 2014; Pradel *et al*., 2017).

The availability of primary (N, P, K) and secondary (S, Ca, Mg) nutrients and minor elements (B, Mn, Zn, Fe) are required to obtain the best yield potential. Mineral nutrients extracted by potatoes depend on the substrate conditions, the fertilization practice, and the variety sown (Egusquiza, 2014; Mokrani *et al*., 2018; Morales-Hernández *et al*., 2013; Niquin *et al*., 2018).

Peruvian coastal soils are deficient in nitrogen and phosphorus; therefore, the supply of macronutrients is essential. The required amounts of nitrogen, phosphorus, potassium (NPK) are 120 to 180 kg N, 60 to 100 kg of P_2O_5, and 0 to 80 kg of K_2O for potato crops (Egusquiza, 2014).

Nitrogen is the nutrient that most affects the yield and quality of tubers (Alva, 2004; Mokrani, 2018; Oliver, 2017). High doses of N promote foliage growth, but delay the initiation of tuberization and reduce yield and quality by decreasing the percentage of dry matter in the tubers (Alva, 2004; Suárez *et al*., 2006). The potato crop can absorb N in the form of nitrate (NO_3^-) and ammonia (NH_4^+), which depend on the age of the plant and the pH of the soil. However, as the availability of nitrate increases, the plant has higher growth rates (Pumisacho and Sherwood, 2002).

Phosphorus is an essential macronutrient in respiration and photosynthesis plant processes. It is part of nucleoproteins, lipids, and phospholipids. It acts on the roots development and meristematic tissues (Salisbury and Ross, 2000; Pumisacho and Sherwood, 2002; Bernal and Espinosa, 2003). Regarding potassium, a crop with high yields can absorb more than 340 kg ha^{-1} of K_2O (MINAGRI, 2011). In potato cultivation, K is needed for the transport of sugars from leaves to the tubers (Becerra-Sanabria *et al*., 2007).

The national average potato yield was 13.72 t ha^{-1} in 2011, being Lima (23.90 t ha^{-1}) and Arequipa (32.77 t ha^{-1}), the departments that contributed the most (MINAGRI, 2012). By 2016, Peru was ranked as the 14th country in potato production worldwide; however, its productivity level (14.78 t ha^{-1}) is 26% lower than the world average. The regions of Arequipa, Ica, and Lima achieved the best average yields with 33.5, 32.2, and 22.7 t ha^{-1}, respectively (MINAGRI, 2017). However, it was not enough since the national average yield has not increased significantly over the last 20 years. On the Peruvian coast, the increase in potato productivity is due to the improved varieties, good quality seed, and high levels of fertilization. However, the soil salinization levels can negatively affect the high dependence on mineral fertilizers (Marchese, 2015). Therefore, the present study was conducted to evaluate the effect of nitrogen and phosphorus fertilization sources and their interaction on the potato cultivation yield under the soil and climate of Peruvian coast conditions.

MATERIALS AND METHODS

The investigation was carried out at the Lagunas farm, in the town of Vinto Bajo, district and province of Barranca in the department of Lima, located at 49 masl, with geographical coordinates, latitude 10°45'1" and longitude 77°45'1". The soil was sandy loam texture, with slightly alkaline pH (7.53), high amount of $CaCO_3$ (5.25%), low organic matter (0.75%), low phosphorus concentration (6.2 ppm), average potassium content (101 ppm), and 1.31 dS m^{-1} of electrical conductivity (qualified as very slightly saline). According to MINAGRI (2011), the acceptable pH for potato production ranges from 5 to 7.

The maximum temperature occurred during the seedling emergence and the minimum temperature in the flowering stage (Figure 1). Low temperatures promoted the tuberization phase, reaching values of 16.7 °C. The medium temperature during crop development fluctuated between 10.75 °C and 20.05 °C. The temperature

between sowing and germination should range between 18-24 °C and 15-22 °C during the growing period. An optimal temperature during the potato tuber bulking phase is 14-18 °C because lower than 10 °C and upper than 30 °C could inhibit the tuber development (Kim and Lee, 2019; MINAGRI, 2012).

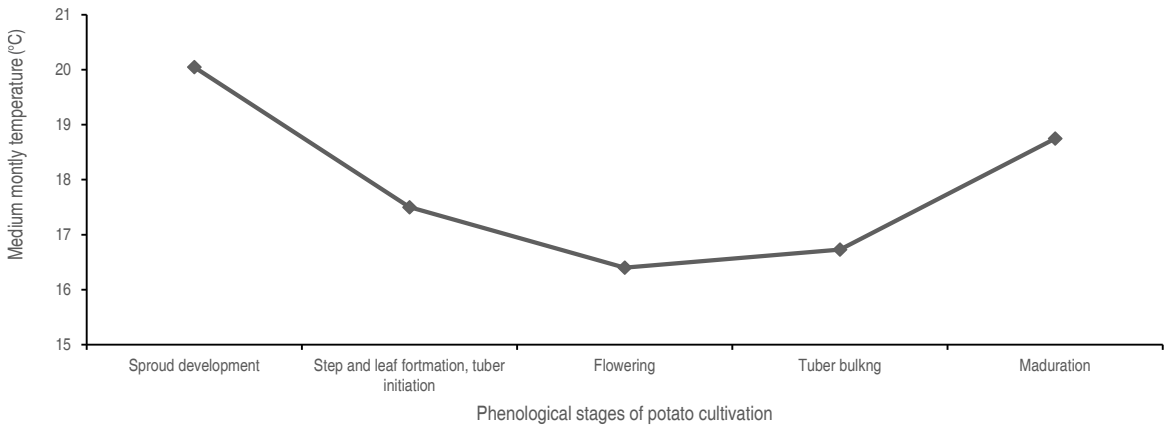


Figure 1. Variation in medium monthly temperature during the experimental phases: Sproud development (30 days after sowing (DAS)); stem and leaf formation and tuber initiation (60 DAS); flowering (75 DAS); tuber bulking (90 DAS); maduration (120 DAS).

The genetic material used for sowing was the improved variety INIA-303 Canchan, widely used in the main potato producing areas of the Peruvian coast since farmers can obtain up to 30 t ha^{-1} in the 120 days (MINAGRI, 2012). A 3×3 factorial randomized block design was established with three nitrogen fertilization sources and three phosphorus fertilization sources as independent factors (nine treatments and four repetitions) (Table 1).

Urea [$CO(NH_2)_2$] with 46% N, Ammonium Nitrate (NO_3NH_4) with 33% N and Ammonium Sulfate $SO_4(NH_4)_2$ with 21% N and 24% S were used as nitrogen fertilization sources. Diammonium Phosphate [$(NH_4)_2HPO_4$] with 18% of N and 46% of P_2O_5, Triple superphosphate $(H_2PO_4)_2$ with 46% P_2O_5, and Monoammonium Phosphate ($NH_4H_2PO_4$) with 11% N and 52% P_2O_5 were used as phosphorus sources (Reetz, 2016).

Table 1. Factors under study.

F1: Nitrogen Sources	F2: Phosphorus Sources	F1×F2 Treatments		
N1: Urea	P1: Diammonium phosphate (DAP)	1: N1P1	4: N2P1	7: N3P1
N2: Ammonium nitrate (AN)	P2: Triple superphosphate (TSP)	2: N1P2	5: N2P2	8: N3P2
N3: Ammonium sulfate (AS)	P3: Monoammonium phosphate (MAP)	3: N1P3	6: N2P3	9: N3P3

The treatments were composed of the combinations of the factors under study (Table 1). The NPK fertilizer formula for all treatments was 276, 166, and 250 kg of N, P_2O_5, and K_2O, respectively. The potato crop usually requires large amounts of K (MINAGRI, 2011).

Fertilization was carried out manually and fractionally. In the sowing period, 60% of P and 36% of N was applied. 46 days after sowing (DAS), the second fertilization was performed, which corresponds to 36% N and 40% P. On 66 DAS, 28% N was added. N was applied fractionally on two stages, according to Egusquiza (2014), 50% N+PK during the sowing period and then at the first hilling period other 50% N.

Tuber number per plant (TNPP), total weight tuber per plant (TWPP), Total (TY), and Commercial (CY) yield were evaluated as the dependent variables (Table 2).

The data obtained for the treatments applied were analyzed, assuming normality and significance of variance by Shapiro-Wilk and Fisher tests (P<0.05),

Table 2. Variables under study and evaluation procedure.

Variables under study	Procedure
Tuber number per plant (TNPP)	Five plants were randomly selected from the central rows of each experimental unit. The number of tubers per plant was then averaged.
Tuber weight per plant (TWPP)	Five plants were chosen at random from the central rows of each experimental unit. The tubers were weighed per plant and expressed in kg per plant.
Total yield (TY) and commercial yield (CY)	Ten linear meters were harvested for each experimental unit, and the yield was expressed in t ha^{-1}. Commercial categories was taken into consideration and expressed in t ha^{-1}.

respectively. Once these assumptions were verified, data were subjected to a two-way ANOVA. Mean values were compared using the Duncan test (P<0.05). All analyses were performed by the statistical software Infostat (Di Rienzo *et al*., 2011).

RESULTS AND DISCUSSION

Nitrogen sources, phosphorus sources, and their respective interaction were highly significant (P<0.01) for total yield and tuber weight per plant; whereas, for commercial yield and the number per plant, the nitrogen sources, phosphorus sources, and their respective interaction were significant at P<0.05. The interaction of the fertilization sources also presented an effect on the variables. Therefore, there was no influence of an uncontrolled factor variation in the experiment (Table 3).

Table 3. Values of F, significance, and coefficients of variation (CV) for total yield Commercial yield, Tuber number per plant, Tuber weigh per plant, by nitrogen and phosphorus fertilization sources.

Sources of variation	TY	CY	TNPP	TWPP
Nitrogen Sources (N)	40.419**	31.374**	3.034*	0.052**
Phosphorus sources (P)	15.919**	12.928**	2.621*	0.023**
N×P	6.598**	5.674*	2.384*	0.036**
Error	0.665	1.711	0.755	0.002
CV (%)	2.62	4.81	8.44	4.13

*P<0.05; **P<0.01

TY: Total Yield; CY: Commercial Yield; TNPP: Tuber number per plant; TWPP: Tuber weigh per plant; CV: Coefficient of variation.

The highest total yield (TY) was found with the N2P1 (AN+DAP) treatment and was higher than the combinations N3P3 (AS+MAP) and N2P3 (AN+MAP) by 3.03% and 4.71%, respectively. For the nitrogen fertilization sources, it could be most recommended the AN and for phosphorus sources, the DAP and MAP according to the soil and climate conditions of the study (Figure 2).

Yields of the urea combinations with DAP, MAP, and TSP were lower compared to the two combinations described above. It could be due to urea does not respond in alkaline or slightly alkaline soils (Cépeda, 2010). The AN has a better response in coastal conditions because the plant develops better when nitrates are available (Pumisacho and Sherwood, 2002), while AS improves the assimilation of P because its acidifying effect reduces soil pH.

According to (MINAGRI, 2011), in strongly alkaline soils, the availability of minor elements is low, which can affect crop yield. Urea is recommended for acidic and neutral soils because, in limestone soils, significant nitrogen losses can occur due to volatilization (Cépeda, 2010). The acidity index of urea, AN, and AS (-84, -63, and -110, respectively) is a favorable factor for temporarily buffering soil pH. Excessive acidification can affect the availability of

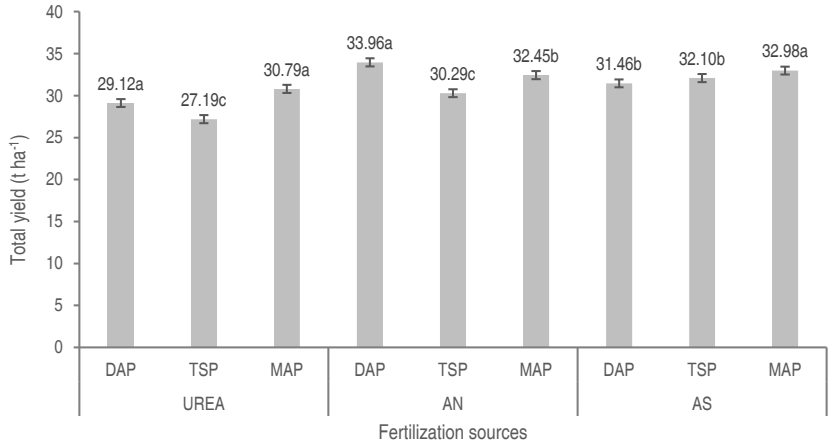


Figure 2. Total yield of INIA-303 Canchan tubers (t ha^{-1}) with the interaction of nitrogen and phosphorus sources. Different letters within the nitrogen source mean a significant difference (P>0.05).

nutrients for plant growth, the levels of phytotoxic elements, the microbial activity, and even the physical conditions of the soil (Cépeda, 2010; Presutti *et al*., 2017). Gutiérrez (2015) stated that the management of nitrogen fertilization, in terms of dose, time, and method of application, influences the yield and quality of the potato tuber. Optimal nitrogen fertilization and irrigation water management are important to improve nitrogen uptake efficiency and minimize N losses (Alva, 2004). The management of these factors is a challenge in the different soils where potatoes are grown, which are generally vulnerable to water leaching and soluble nutrients (Sifuentes *et al*., 2015). In general, fertilizers with a high content of ammonium nitrogen origin can acidify soils when they are applied repeatedly. Microorganisms in the soil convert the nitrogen from ammonium (NH_4^+) to nitrate (NO_3^-), thus releasing ions H^+, which acidify the soil (Wadas and Dziugiel, 2015).

For commercial yield (CY), the highest value was achieved by AN+DAP, which was statistically similar to the AN+MAP. AN+TSP was the lowest value (26.10 t ha^{-1}) and is different from other treatments. The combination AS+MAP achieved the best value (29.18 t ha^{-1}), but it did not have a statistical significance (Figure 3). The combinations of urea with DAP, TSP, and MAP showed a lower yield, possibly due to the acidity index of the fertilizers used.

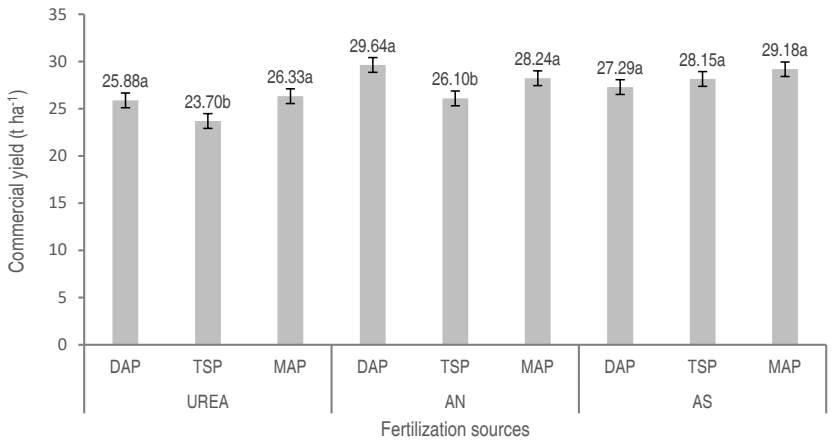


Figure 3. Interaction of nitrogen and phosphorus sources in the commercial yield of INIA-303 Canchan tubers (t ha^{-1}). Different letters within the nitrogen source mean a significant difference (P>0.05).

According to MINAGRI (2017), the average yield at the coastal level was 25 t ha^{-1}. Both the total yield and commercial yield were above the national average (14.5 t ha^{-1}). Soil fertility is a quality resulting from the interaction between the physical, chemical, and biological characteristics of the soil, and it consists of the capacity to provide necessary conditions for plant growth and development (Egusquiza, 2014). Soil pH has an indirect influence on chemical processes, nutrient availability, biological processes, and microbial activity (MINAGRI, 2011).

The highest TNPP and TWPP were found by the AN+DAP (Figure 4 and Figure 5). AN+DAP combination, for the TNPP variable, was higher than AN+MAP and AS+MAP treatments with 4.90% and 15.72%, respectively. AN+DAP was higher than AS+MAP and AN+MAP with 3.03% and 4.71%, respectively for the TWPP variable. The highest TNPP average was 11.85, while the highest TWPP was 1,214 kg per plant. According to Egusquiza (2014) and INIA (2012), the cultivar INIA-303 Canchan has 20 tubers per plant as the average TNPP, while the average TWPP can reach 1 kg per plant.

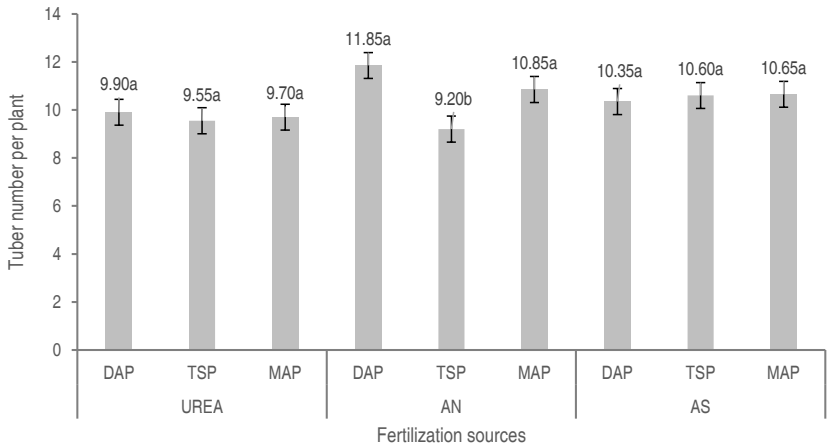


Figure 4. Effect of the combination of nitrogen and phosphorus sources in the variable tuber number per plant yield (TNPP). Different letters within the nitrogen source mean a significant difference (*P*>0.05).

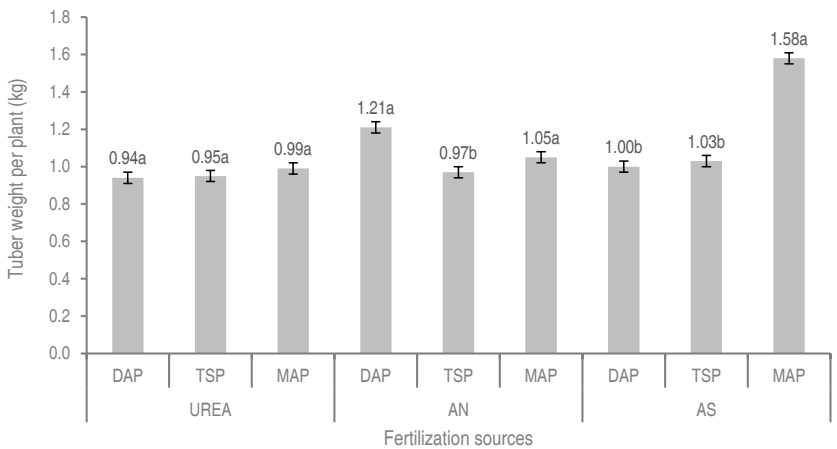


Figure 5. Effect of the combination of nitrogen and phosphorus sources in the tuber weight per plant yield (TWPP). Different letters within the nitrogen source mean a significant difference (*P*>0.05).

CONCLUSIONS

The INIA-303 Canchan cultivar showed positive response to the combinations of nitrogen and phosphorus fertilization sources under the soil and climate conditions of the Peruvian coast. The total and commercial yield, tuber number per plant and tuber weigh per plant were significantly affected by the interaction of nitrogen and phosphorus sources. The highest total yield, commercial yield, number and weight of tubers per plant were found with the combination of ammonium nitrate+diamonic phosphate. The three most recommended mixtures for the fertilization of the INIA-303 Canchan potato variety sown in Peruvian coast, to achieve yields between 32.45 t ha^{-1} and 33.98 t ha^{-1}, are ammonium nitrate+diammonium phosphate, ammonium sulfate+monoammonium phosphate, and ammonium nitrate+monoammonium phosphate.

REFERENCES

Alva L. 2004. Potato Nitrogen Management. Journal of Vegetable Crop Production 10(1): 97-132. doi: 10.1300/J068v10n01_10

Becerra-Sanabria LA, Navia de Mosquera SL y Ñústez-López CE. 2007. Efecto de niveles de fósforo y potasio sobre el rendimiento del cultivar 'Criolla Guaneña' en el departamento de Nariño. Revista Latinoamericana de la Papa 14(1): 51-60.

Bernal J y Espinosa J. 2003. Manual de nutrición y fertilización de pastos. International Plant Nutrition Institute, Quito, Ecuador. 94 p.

Cépeda J. 2010. Fertilidad de suelos I. Escuela de Agronomía. En: AGRO 6505 – Fertilidad de Suelos Avanzada, http://academic.uprm.edu/dsotomayor/agro6505/Cepeda_CAPITULOS_1-7_511.pdf.

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M y Robledo CW. 2011. InfoStat versión 2011. Universidad Nacional de Córdoba, Argentina. http://www.infostat.com.ar.

Egusquiza R. 2014. La papa en el Perú. Segunda edición. Universidad Nacional Agraria La Molina. Lima, Perú.

Fernandes AM, Soratto RP, Moreno LA and Evangelista RM. 2015. Effect of phosphorus nutrition on quality of fresh tuber of potato cultivars. Bragantia 74(1): 102-109. doi: 10.1590/1678-4499.0330

Fonseca C, Labarta R, Mendoza A, Landeo L y Walker T. 1996. Impacto económico de la variedad Canchán INIAA, de alto rendimiento y resistente al tizón tardío, en el Perú. Centro Internacional de la Papa, Lima. 33 p.

Gutiérrez R. 2015. Eficiencia de la fertilización nitrogenada en el cultivo de papa *Solanum tuberosum* cv Yungay en Colpar, distrito de Quilcas-Huancayo. (Tesis de Pregrado) Universidad Nacional del Centro del Perú. Huancayo, Perú. 87p.

INIA. 2012. Papa INIA-303 Canchán. En: https://www.inia.gob.pe/wp-content/uploads/investigacion/programa/sistProductivo/variedad/papa/INIA_303.pdf . Consulta: Agosto, 2020.

Kim YU and Lee BW. 2019. Differential mechanisms of potato yield loss induced by high day and night temperatures during tuber initiation and bulking: Photosynthesis and tuber growth. Frontiers in Plant Science 10: 300. doi: 10.3389/fpls.2019.00300

Marchese A. 2015. Estudio físico y químico de suelos agrícolas para la estimación del nivel de salinización en el sector bajo de San Pedro de Lloc. (Tesis de Pregrado). Pontificia Universidad Católica del Perú. Lima, Perú. 109p.

MINAGRI 2011. Cadena agroproductiva de papa manejo y fertilidad de suelos. Dirección General de Competitividad Agraria, Lima. 50 p.

MINAGRI 2012. La papa: Principales aspectos de la cadena agroproductiva de la papa. Dirección General de Competitividad Agraria, Lima. 35 p.

MINAGRI 2017. Papa: Características de la producción nacional y de la comercialización en Lima Metropolitana. Dirección General de Políticas Agrarias Dirección, Lima. 13 p.

Mokrani K, Hamdi and Tarchoun N. 2018. Potato (*Solanum Tuberosum* L.) Response to nitrogen, phosphorus and potassium fertilization rates. Communications in Soil Science and Plant Analysis 49(11): 1314-1330. doi: 10.1080/00103624.2018.1457159

Morales-Hernández JL, Hernández-Martínez J y Rebollar-Rebollar S. 2013. Rendimiento de papa con fuentes de fertilización mineral en un Andosol del Estado de México. Revista Mexicana de Ciencias Agrícolas 4(6): 881-893. doi: 10.29312/remexca.v4i6.1156

Niquin-Alayo E, Vergara-Moreno E y Calderón-Niquín M. 2018. FERTIDIF: software para la planificación de fertilización agrícola basado en optimización lineal con costos difusos. Scientia Agropecuaria 9(1): 103 – 112. doi: 10.17268/sci.agropecu.2018.01.11

Oliver J. 2017. Rendimiento de dos variedades de papa (*Solanum tuberosum* L.) con la aplicación de tierra negra y fertilizantes inorgánicos. Revista de Investigación e Innovación Agropecuaria y de Recursos Naturales 4(2): 56-62.

Pradel W, Hareau G, Quintanilla L y Suárez V. 2017. Adopción e impacto de variedades mejoradas de papa en el Perú: Resultado de una encuesta a nivel nacional (2013). Centro Internacional de la Papa. Lima. 48 p.

Presutti M, Bennardi D, Frias Calvo A y Vázquez M. 2017. Acidificación de los suelos de la provincia de Buenos Aires y su necesidad de enmienda calcárea. Revista de Divulgación Técnica Agropecuaria, Agroindustrial y Ambiental Facultad de Ciencias Agrarias. UNLZ 4(1): 67-76.

Pumisacho M y Sherwood S. 2002. Cultivo de la papa en Ecuador. Instituto Nacional Autónomo de Investigaciones Agropecuarias, Quito. 232p.

Reetz H. 2016. Fertilizers and their efficient use. First edition. IFA, Paris, 110 p.

Salisbury F y Ross C. 2000. Fisiología de las plantas. Ediciones Paraninfo SA, Madrid. 480 p.

Sifuentes E, Ruelas J, Macías J, Talamantes, I, Palacios C and Valenzuela B. 2015. Fenología y tiempo en el manejo del riego y fertilización del cultivo de papa. Biotecnia 17(3): 42-48.

Suárez L, Giletto C, Rattín J, Echeverría H y Caldiz D. 2006. Efecto del nitrógeno sobre el rendimiento y la calidad de tubérculos en papa para industria. Informaciones Agronómicas del Cono Sur 32(5): 19-21.

Wadas W and Dziugiel T. 2015. Effect of complex fertilizers used in early crop potato culture on loamy sand soil. Journal of Central European Agriculture 16(1): 23-40. doi: 10.5513/JCEA01/16.1.1536.

Research article

http://www.revistas.unal.edu.co/index.php/refame

Revista Facultad Nacional de Agronomía

Gamma ray irradiation (Co^{60}) of lulo with and without thorns calluses and seedlings (*Solanum quitoense* Lam.) produced *in vitro*

Irradiación con rayos gamma (Co^{60}) de callos y plántulas de lulo con y sin espinas (*Solanum quitoense* Lam.) producidas *in vitro*

doi: 10.15446/rfnam.v73n3.82362

Hernando Criollo-Escobar[1]* and Johanna Muñoz-Belalcázar[1]

ABSTRACT

The cultivation of lulo de Castilla (*Solanum quitoense* Lam.) in Colombia is subject to a series of sanitary problems, which has forced many producers to abandon the crop as a result of the total loss of plantations or to transfer the crop to new areas. It is necessary to implement breeding programs in order to produce varieties that are tolerant to the limiting problems. Since these programs require broad genetic variability in the progenitors, the present study aimed to evaluate the possibility of inducing *in vitro* variability in explants subjected to different doses of gamma radiation using a Co^{60} source. The evaluated radiation doses were 0 Gy, 15 Gy, 30 Gy, 45 Gy, and 60 Gy in calluses induced with cotyledonary leaves and in seedlings from *in vitro* cultures of lulo with and without thorns. The survival and regeneration potential were also evaluated. The calluses were the explants that showed the highest survival, and the lulo seedlings without thorns were the most radiosensitive with a mortality of 100% at a dose of 30 Gy. The lulo seedlings with thorns had 100% mortality at a dose of 45 Gy. The irradiated lulo seedlings with thorns had a greater regeneration capacity than the lulo without thorns, with 1.52 seedling per explant and 1.12 seedling per explant, respectively, and the RAM markers showed genetic variability in all the irradiation treatments.

Keywords:
Genetic variation
Mutagenesis
Naranjilla
RAM
Survival

RESUMEN

El cultivo de lulo de Castilla (*Solanum quitoense* Lam.) en Colombia está sometido a una serie de problemas principalmente de índole sanitario, que han obligado a muchos productores al abandono del cultivo por la pérdida total de las plantaciones o al traslado del cultivo a zonas nuevas, por lo cual se hace necesario la implementación de programas de mejoramiento para la búsqueda de variedades tolerantes a los problemas limitantes. Teniendo en cuenta que estos programas requieren una amplia variabilidad genética de sus progenitores, el presente estudio se orientó a evaluar la posibilidad de inducir variabilidad *in vitro*, de explantes sometidos a diferentes dosis de radiaciones gamma utilizando una fuente de Co^{60}. Se evaluaron dosis de radiación con 0 Gy, 15 Gy, 30 Gy, 45 Gy y 60 Gy en callos inducidos a partir de hojas cotiledonares y en plántulas provenientes de cultivos *in vitro* de lulo con y sin espinas. La supervivencia y el potencial de regeneración también fueron evaluados. Los callos fueron los explantes que presentaron mayor supervivencia y las plántulas de lulo sin espinas las más radiosensitivas con una mortalidad del 100% a una dosis de 30 Gy; las plántulas de lulo con espinas tuvieron una mortalidad del 100% con una dosis de 45 Gy. Las plántulas irradiadas de lulo con espinas tuvieron mayor capacidad de regeneración que las de lulo sin espinas con 1,52 plántulas por explante y 1,12 plántulas por explante, respectivamente; además, los marcadores RAM mostraron variabilidad genética con todos los tratamientos de irradiación estudiados.

Palabras clave:
Variación genética
Mutagénesis
Naranjilla
RAM
Supervivencia

[1] Facultad de Ciencias Agrícolas. Universidad de Nariño. Calle 18 No. 50-02. CP 520002, Pasto, Nariño, Colombia.
* Corresponding author: <hcriolloescobar@gmail.com>

Received: February 28, 2020; Accepted: July 17, 2020.
Rev. Fac. Nac. Agron. Medellín 73(3): 9263-9271. 2020

ISSN 0304-2847 / e-ISSN 2248-7026

The cultivation of lulo in Colombia is of great importance, and the international potential of this fruit is promising. The increase in the harvested area went from 7,039 to 8,655 ha between 2010 and 2016. In production, it rose from 59,091 to 78,610 t in the same period, demonstrating the interest of producers and the economic advantages that this fruit offers (Agronet, 2018). In addition, the recent free trade agreements (FTAs) signed with several countries around the world opened new markets for Colombian agriculture, which requires strong technological boosts in crops to make Colombia highly competitive, as seen in the case of the lulo.

Lulo producers in Colombia face the susceptibility of cultivars to pests and diseases, farm heterogeneity, and reduction of vegetative and reproductive development. Agronomic problems that limit productivity and, in some cases, cause total crop loss (Lobo, 2006; Cruz *et al*., 2007; Muñoz, 2013). Despite the losses caused by these problems, the cultivation area and production increased by 7.58% and 3.34%, respectively, in Nariño (Colombia) between 2010 and 2016 (Agronet, 2018). By searching suitable culturable areas, the low technological level of lulo farming systems has caused deforestation, making it necessary to rethink current cultivation techniques in order to avoid environmental problems (Muñoz, 2013).

These drawbacks mean that breeding programs aimed at increasing productivity are needed. It pursues lulo varieties tolerant to the major diseases and greater efficiency in transforming light into chemical energy, along with better distribution of carbohydrates (Angulo, 2006).

In Colombia, minimal efforts in lulo breeding have been geared toward obtaining cultivars resistant or tolerant to pathogens and pest, such as *Fusarium* sp., *Phytophthora infestans*, *Neolucinodes elegantalis*, and *Meloidogyne* sp. It has been necessary to resort to related species and obtain hybrids with resistance, but with lower quality, since all lulo varieties show susceptibility (Betancourth *et al*., 2005; Muñoz, 2013). Despite its low domestication, the lulo has stability in specific niches and restrict general adaptability (Lobo, 2006).

Genetic variability is essential in plant breeding programs, it can be a definitive solution in crop breeding, especially in crops with limited variability (Subramanian, 2011; Rimieri, 2017). The induction of mutations is a widely used method to produce genetic variability, but it is necessary to evaluate the effectiveness and efficiency of each technique for the frequency of desirable mutants before considering one a potent tool for the improvement of plants (Prina *et al*., 2010).

Artificial mutagenic agents can be physical or chemical. Treatments with ionizing radiation produce high degrees of chromosomal damage with breaks in the DNA chain and physiological effects that cause cell death and sterility in first-generation plants. Currently, the most commonly used radiation for plant breeding purposes includes x-rays and gamma rays (Sonone *et al*., 2010; Prina *et al*., 2010; Velmurugan *et al*., 2010; Silvera, 2017). However, the mutation rate can also be increased by *in vitro* cultures (somaclonal variation). It is possible to select plants by obtaining agronomically useful mutants and combine mutation techniques with *in vitro* propagation (Patade *et al*., 2008).

The improvement of the tolerance to water stress conditions of many crops has been achieved with *in vitro* selection of mutants obtained with radiation or chemical methods. These procedures have been tested on multiple crops, such as potatoes (Velmurugan *et al*., 2010), peanuts (Sonone *et al*., 2010), soybeans (Satpute and Fultambkar, 2012; Kavithamani *et al*., 2010; Ortiz *et al*., 2008), rice (Baloch *et al*., 2004), sorghum (Jayaramachandran *et al*., 2010), cherries (Roman *et al*., 2009), sunflower (Kumar and Ratnam, 2010), sugar cane (Singh, 1993; Desai, 2006), among others. The improvement of crops with genetic mutations has also been successful in obtaining new colors and shapes in ornamental plants, and many mutants are induced by gamma radiation and other mutagens that are currently marketed (Datta *et al*., 2005; Matsumura *et al*., 2010; Sahariya *et al*., 2017)

Mutagenic induction with chemicals and radiation has been successfully used to obtain desirable agronomic characteristics. The 89% of mutant varieties have been obtained with irradiation methods, from which 64% were obtained using gamma radiation (Patade, 2008).

According to Predieri and Zimmerman (2001) and Mostafa *et al.* (2015), the effectiveness and efficiency of irradiation depend on the dose used, the genotype, and the type of irradiated explant. Therefore, the present study aimed to

carry out an *in vitro* evaluation of the effect of Co^{60} gamma ray irradiation on the somaclonal variation of lulo (*Solanum quitoense* Lam) with and without thorns.

MATERIALS AND METHODS

This study was carried out in the Tissue Laboratory of the Biology program of the Faculty of Sciences at the Universidad Nacional de Colombia (Medellín Headquarters); the gamma radiation was carried out in the nuclear reactor of the Instituto Colombiano de Geología y Minería (INGEOMINAS), using a Co^{60} source.

The plants of lulo with and without thorns were obtained with three subcultures in an MS medium and calluses produced with foliar segments of lulo with thorns in an MS medium supplemented with ANA (6.0 mg L^{-1}) and sucrose at 9%, which were incubated in darkness for 45 days, and then were irradiated.

The plants and calluses were cultivated in Petri dishes (Ø=9 cm) in an MS medium before being irradiated in a pilot irradiation plant, with an activity of 10000 curies and a cobalt60 source; the irradiation doses were 0 Gy (Control), 15 Gy, 30 Gy, 45 Gy, and 60 Gy. Immediately after the treatment, the plants and calluses were transplanted to containers with a fresh MS culture medium. A completely randomized design, with a bifactorial arrangement, was used to analyze the irradiated plants' prolificacy and survival. The factors corresponded to the two varieties of lulo (Factor A) and the five doses of irradiation (Factor B). Each irradiated treatment (plants and callus) consisted of eight repetitions (Petri dishes), with four explants each.

To analyze the effect of the irradiation on the genetic fidelity, foliar samples were taken from the live explants after six months for DNA extraction following the methodology of Dellaporta *et al.* (1983) at a concentration of 10 ng μL^{-1}, based on dilutions of phage lambda DNA (Sigma, St. Louis, MO, USA).

In the PCR, eight primers were tested, and seven were analyzed, considering that the CGA primer showed no amplification. The primers used in the RAM technique to determine the effect of the gamma irradiation on the lulo plants and calluses are presented in Table 1.

Table 1. Primers and sequences used in the RAM technique to determine the genetic stability of the lulo seedlings and calluses irradiated with gamma rays.

Primer	Sequence 5' - 3'
CT	DBDCTCTCTCTCTCTCTC
CGA	DHBCGACGACGACGACGA
CA	DBDACACACACACACACACA
AG	HBHAGAGAGAGAGAGAGAG
TG	HVHTGTGTGTGTGTGTGTGT
CCA	DDBCCACCACCACCA
GT	VHV GTG TGT GTG TG
ACA	BDB ACA ACA ACA ACA ACA

Designations used for degenerated sites: H (A or T or C); B (G or T or C); V (G or A or C) and D (G or A or T).

The hybridization and amplification conditions were established according to the selected primer (Table 2). The products were separated and visualized in 7% polyacrylamide gels (37:1 acrylamide-bisacrylamide) ran at 160 V for 1 h and 10 min, and stained with ethidium bromide and silver salts as described by Sambrook *et al.* (1989).

The observation of the gels provided a binary matrix for presence (1) and absence (0). Based on this matrix, descriptive analyses were carried out, and the genetic distances and similarities were established. The unbiased minimum distance criterion (Nei, 1978) was used for the classification analysis following the UPGMA method.

Table 2. Hybridization and amplification conditions for the RAM technique with the selected primers.

Cycle	Temperature (°C)			Time			Stage
	Primer						
	AGCAACA	TGCTCCA	GTCGA	AGCAACA	TGCTCCA	GTCGA	
1		95			5 min		Initial denaturation
2		95			30 s		Denaturation
3	50	55	58		45 s		Hybridization
4		72			2 min		Amplification
5				37 cycles from step two			
6		72			7 min		Final amplification
7		16			5 min		End

These analyses were carried out using TFPGA (Tools for Population Genetic Analysis), as described by Miller (1997).

RESULTS AND DISCUSSION

Survival

The analysis of variance for the percentage of live explants two months after the irradiation showed highly significant differences between the lulo varieties, between the irradiation doses and for the variety×dose interaction (Table 3). At the variety level, the lulo with thorns presented greater survival at the higher radiation doses, requiring an approximate dose of 28 Gy to cause 50% mortality. The lulo variety without thorns proved to be more susceptible to irradiation, with the same level of mortality at a radiation dose of 19 Gy (Figure 1). In general, the plants submitted to high doses presented foliar chlorosis, necrosis of the basal regions of the petioles, leaf fall, growth arrest, and death.

Table 3. Analysis of variance for the survival and regeneration capacity variables of lulo seedlings subjected to different doses of radiation with gamma rays.

Source	Sum of Squares			
	Survival		Regeneration	
	DF	SS	DF	SS
Variety	1	3781.25 **	1	1.95 **
Dose	4	121140.62 **	2	14.92 **
Variety×Dose	4	5828.12 **	2	0.84 *
Error	70		42	

**Significant differences at 99% probability
* Significant differences at 95% probability

The reaction among the lulo varieties has been observed in other species, such as soybeans (Satpute and Fultambkar, 2012), mung bean (Singh, 2007), rice (Baloch, *et al*., 2004), beans (Rocha *et al*., 2010) and pears (Predieri and Zimmerman, 2001). These differences can be attributed to the differential sensitivity of plants to radiation, because their adaptation to light (WT) and understory (WOT) conditions, may affect physiological properties at the cellular and nuclear level (Baloch *et al*., 2004; Rocha *et al*., 2010); an examination of the leaf histology indicates that the two varieties present very similar characteristics (Medina *et al*., 2008)

Multiplication capacity

The regeneration capacity of the plants from the initial explants after irradiation presented statistical differences

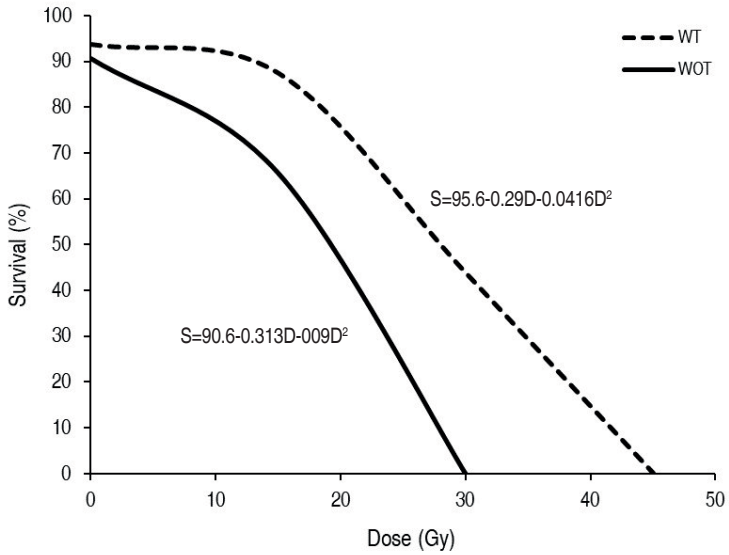


Figure 1. Survival percentage of lulo seedlings (*Solanum quitoense* Lam.) with thorns (WT) and without thorns (WOT) subjected to different doses of gamma radiation with Co^{60}.

between the varieties, irradiation dose and in the varietyxdose interaction (Table 3). At the variety level, the lulo with thorns had a greater multiplication capacity (1.52 seedlings per explant) than the thornless variety (1.12 seedlings per explant), confirming that the latter is more radiosensitive since, at the control level (0 Gy), they had a similar behavior (2.33 seedlings per explant and 2.37 seedlings per explant, respectively). The ability of the initial explants to regenerate plants in the first 30 days after irradiation was correlated with the intensity; as the irradiation dose increased, the prolificacy of the explants was markedly reduced (Figure 2). The radiation-induced necrosis caused this result at the meristematic level, which caused general growth arrest that prevented or reduced the formation of appropriate phytomers for multiplication.

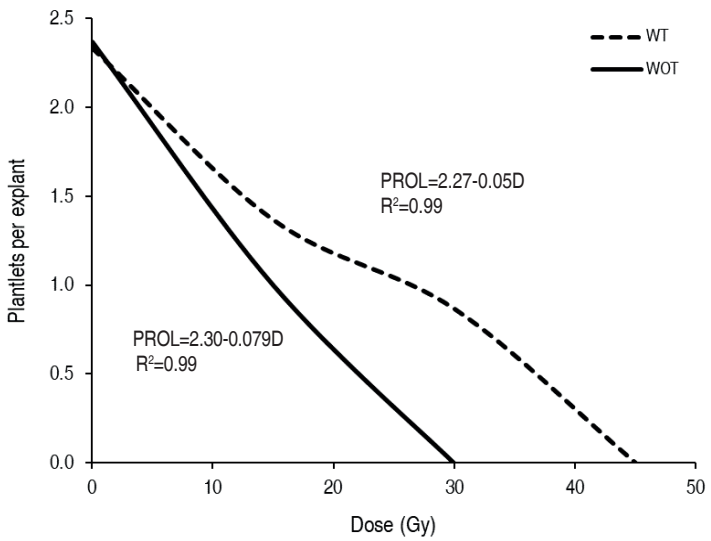


Figure 2. Effect of the of Co^{60} gamma radiation dose on the regeneration capacity (plantlets per explant) of the plants in explants of two lulo varieties (*Solanum quitoense* Lam) with thorns (WT) and without thorns (WOT).

Genetic Variability

The band count for the seven primers showed 115 bands for the irradiated seedlings and 114 for the irradiated calluses; the number of bands was between 12 for the TG primer and 21 for the ACA primer (Figure 3).

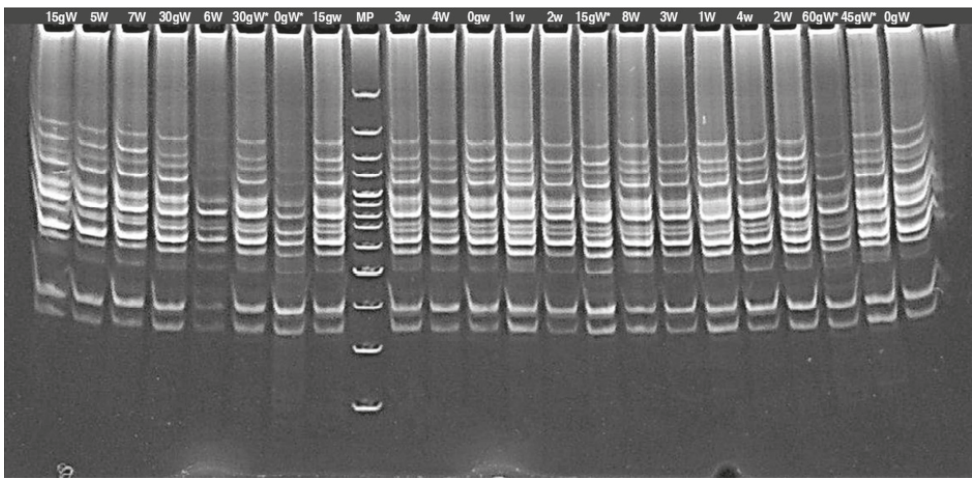


Figure 3. Banding patterns generated with RAM primer in lulo (*Solanum quitoense* Lam.) calluses with thorns (*) and in lulo seedlings with (W) and without (w) thorns, irradiated with gamma rays. g: indicate the irradiation doses; MP: marker.

The lulo calluses irradiated with different doses of gamma rays showed average unbiased heterozygosity of 0.22 and polymorphism of 50%. The analysis of the genetic distances provided values between 0.40 (between 0 Gy and 30 Gy) and 0.17 (between 0 Gy and 60 Gy). Likewise, the control treatment (without irradiation) presented similar values of 0.66 and 0.84 when compared with the irradiation doses of 30 Gy and 60 Gy, respectively (Table 4). The observed variability confirmed the effectiveness of gamma radiation in the induction of genetic variants at the level of the treated lulo explants (callus) and the sensitivity of the species to gamma radiation. This sensitivity depends on factors such as the physiological properties of the cells and nuclei, DNA content, chromosome size and ploidy level (Baloch *et al.*, 2004, Subramanian *et al.*, 2011).

Table 4. Matrix of genetic distance (above the diagonal) of Nei (1978) and similarity (below the diagonal) obtained in lulo with thorns calluses irradiated with Co^{60} gamma rays.

Treatment	1(0 Gy)	2(15Gy)	3(30Gy)	4(45Gy)	5(60Gy)
1	-	0.34	0.40	0.34	0.17
2	0.71	-	0.31	0.25	0.27
3	0.66	0.72	-	0.16	0.37
4	0.71	0.77	0.85	-	0.27
5	0.84	0.76	0.68	0.76	-

The cluster analysis using the UPGMA criterion with Nei's distances (1972) formed four nodes. Node 1, with a distance of 0.16, contained the doses 30 Gy and 45 Gy, which, along with the 15 Gy dose, formed the second node, while the control dose (0 Gy) and the maximum dose of 60 Gy formed a third group with a distance of 0.17. All treatments were included in the fourth node (Figure 4).

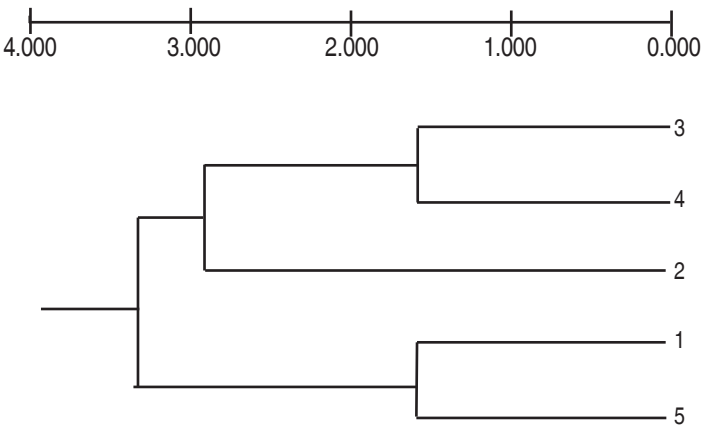


Figure 4. Dendogram of the genetic variability of calluses induced with lulo cotyledonary leaves and irradiated with Co^{60} gamma ray doses (Based on Nei's distances, combined data of seven RAM primers and UPGMA classification criteria; 1 = 0 Gy, 2 = 15 Gy, 3 = 30 Gy, 4 = 45 Gy, 5 = 60 Gy).

The analysis of the irradiation effect on lulo seedlings with and without thorns revealed unbiased heterozygosity of 0.13 and polymorphism of 31.03%; heterozygosity estimates the probability that two randomly drawn alleles from a set of genes in a population are different. The greatest genetic distance (0.22) was observed between the lulo with and without thorns controls, with a similarity of 0.80. The distance between the lulo with thorns control and the doses 15 Gy and 30 Gy was 0.15 and 0.18, with a similarity of 85% and 83%, respectively. There was a genetic distance of 0.15 and a similarity of 85% between the lulo without thorns control and the dose 15 Gy. The heterogeneity of responses to radiation between species and between genotypes of the same species was reported by Arena *et al.* (2017), attributed mainly to differences between DNA contents (Subramanian *et al.*, 2011); however, the IAEA (1977) stated that radiosensitivity differences between genotypes of the same species are much lower than between other species.

The cluster analysis formed four groups. The treatments with 15 Gy on lulo without thorns and with 30 Gy on lulo with thorns formed group one, located at a distance of 0.13, which, along with the control treatment without thorns, formed group two at a distance of 0.17. Group three contained the treatments 0 Gy and 15 Gy with thorns at a distance of 0.14, and group four included all treatments at a distance of 0.18 (Figure 5).

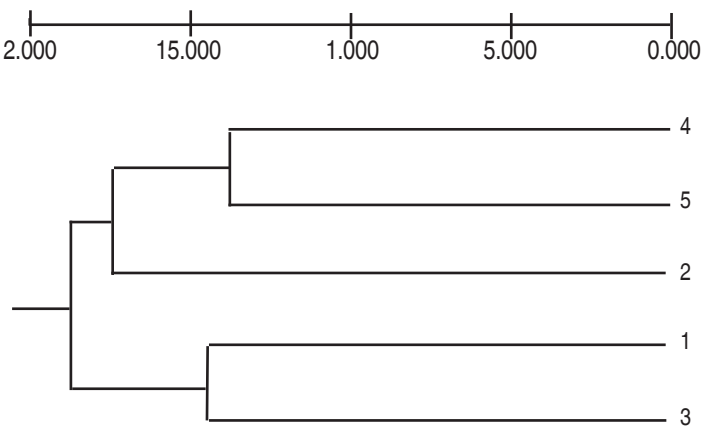


Figure 5. Dendogram of the genetic variability of lulo seedlings with (CE) and without thorns (SE) irradiated with different Co^{60} gamma ray doses (Based on Nei's distances, combined data of seven RAM primers and UPGMA classification criteria; 1 = 0Gy CE, 2 = 0Gy SE, 3 = 10Gy CE, 4 = 15Gy SE, 5 = 30Gy SE).

CONCLUSIONS

The Gamma ray irradiation of calluses induced from cotyledonary leaves of lulo and seedlings regenerated from lulo with and without thorns, generates genetic variability. The survival of the explants at the different doses of gamma radiation showed that the calluses were less radiosensitive (28 Gy, 50% mortality) and the lulo seedlings without thorns, were the most sensitive to radiation (19 Gy, 50% mortality). The ability of the irradiated explants (seedlings) to regenerate plants was inversely proportional to the dose of gamma rays received; the effects of the radiation were genotype-dependent.

ACKNOWLEDGMENTS

The authors express their gratitude to the Dirección de Asuntos Nucleares del Ministerio de Minas y Energía, the Universidad Nacional de Colombia, Bogotá and Palmira, Dr. Margarita Perea, Dr. Jaime Eduardo Muñoz, and the Grupo de Investigación en Producción de Frutales Andinos of the Universidad de Nariño.

REFERENCES

Agronet. 2018. Area, producción y rendimiento nacional por cultivo. http://www.agronet.gov.co/estadistica/Paginas/default.aspxConsulta: noviembre, 2018.

Angulo R. 2006. Lulo: el cultivo. Fundación Universidad de Bogotá Jorge Tadeo Lozano, Bogotá. 100 p.

Arena C, Turano M, Hay Mele B, Cataletto P, Furia M, Pugliese M and De Micco V. 2017. Anatomy, photochemical activity, and DNA polymorphism in leaves of dwarf tomato irradiated with X-rays. Biologia Plantarum 61(2):305-314. doi: 10.1007/s10535-016-0668-5

Baloch AW, Soomro AM, Bughio HR, Bughio MS, Mohammed T and Mastoi NN. 2004. Gamma irradiation induced chlorophyll mutations in rice (*Oryza sativa* L.). SAARC Journal of Agriculture 2: 257-261.

Betancourth C, Zambrano M y Narvaez C. 2005. Reacción de diferentes genotipos de lulo (*Solanum quitoense*) al ataque de *Fusarium oxysporum*. Revista de Ciencias Agrícolas 22(1-2):79-90.

Cruz P, Acosta K, Cure JR y Rodríguez D. 2007. Desarrollo y fenología del lulo *Solanum quitoense* var. *septentrionale* bajo polisombra desde siembra hasta primera fructificación. Agronomía Colombiana 25(2): 288-298.

Datta SK, Misra P and Mandal AKA. 2005. *In vitro* mutagenesis a quick method for establishment of solid mutant in chrysanthemum. Current Science 88: 155-158.

Desai N, Joseph D, Suprasanna P and Bapat V. 2006. Study of elemental variations during somatic embryogenesis in sugarcane using photon induced X-ray probe. Nuclear Instruments and Methods in Physics Research 252(2): 299-302. doi: 10.1016/j.nimb.2006.08.009

IAEA - International Atomic Energy Agency. 1977. Manual on mutation breeding. Second edition. Technical reports series No. 119, Vienna, Austria. 288 p.

Jayaramachandran M, Kumaravadivel N, Eapen S and Kandasamy G. 2010. Gene Action for yield attributing characters in segregating generation (M2) of Sorghum (*Sorghum bicolor* L.). Electronic Journal of Plant Breeding 1(4): 802-805.

Kavithamani D, Kalamani A, Vanniarajan C and Uma D. 2010. Development of new vegetable soybean (*Glycine max* L. Merill) mutants with high protein and less fibre content. Electronic Journal of Plant Breeding 1(4): 1060-1065.

Kumar P and Ratnam S. 2010. Mutagenic effectiveness and efficiency in varieties of sunflower (*Helianthus annuus* L.) by separate and combined treatment with gamma-rays and sodium azide. African Journal of Biotechnology 9(39): 6517-6521.

Lobo M. 2006. Recursos genéticos y mejoramiento de frutales andinos: una visión conceptual. Ciencia y Tecnología Agropecuaria 7(2): 40-54. doi: 10.21930/rcta.vol7_num2_art:68

Matsumura A, Nomizu T, Furutani F, Hayashi K, Minamiyama Y and Hase Y. 2010. Ray florets color and shape mutants induced by $^{12}C^{5+}$ ion beam irradiation in chrysanthemum. Scientia Horticulturae 123: 558-561. doi: 10.1016/j.scienta.2009.11.004

Medina C, Sanchez D, Camayo G, Lobo M, y Martínez E. 2008. Anatomía foliar comparative de materiales de lulo (*Solanum quitoense* Lam.) con y sin espinas. Ciencia y Tecnología Agropecuaria 9(1): 5-13.

Mostafa HHA, Wuan H, Shen D, Qiu Y, and Li X. 2015. Sprout differentiation and mutation induction of garlic (*Allium sativum* L.) callus exposed to gamma radiation. Plant Growth Regulation 75: 465-471. doi: 10.1007/s10725-014-0009-7

Muñoz JA, Rodríguez LF y Bermúdez LT. 2013. Análisis de la competitividad del sistema de producción de lulo (*Solanum quitoense* Lam.) en tres Municipios de Nariño. Revista Colombiana de Ciencias Hortícolas 7(2): 173-185. doi: 10.17584/rcch.2013v7i2.2233

Ortiz R, De la Fé C y Ponce M. 2008. Incasoy-36: variedad de soya obtenida en cuba a partir de la inducción de mutaciones con los rayos gamma de ^{60}Co. Cultivos Tropicales 29(3):73.

Patade VY, Suprasanna P and Bapat VA. 2008. Gamma Irradiation of Embryogenic Callus Cultures and *In vitro* Selection for Salt Tolerance in Sugarcane (*Saccharum officinarum* L.). Agricultural Sciences in China 7(9): 1147-1152. doi: 10.1016/S1671-2927(08)60158-3

Predieri S and Zimmerman RH. 2001. Pear mutagenesis: *In vitro* treatment with gamma-rays and field selection for productivity and fruit traits. Euphytica 117(3): 217–227. doi: 10.1023/A:1026594103277

Prina A, Landau A, Pacheco M, y Hopp E. 2010. Mutagénesis, TILLING y EcoTILLING. pp. 218-228. En: Levitus G, Echenique V, Rubinstein C, Hopp E y Mroginski L (eds.). Biotecnología y Mejoramiento Vegetal II. ArgenBio, Ediciones Instituto Nacional de Tecnología Agropecuaria, Argentina. 648p.

Rocha F, Coan M, Coimbra J, Bertoldo J, Guidolin A and Kopp M. 2010. Root distribution in common bean populations used in breeding programs. Crop Breeding and Applied Biotechnology 10: 40-47.: doi: 10.12702/1984-7033.v10n01a06

Roman G, Harsan E and Wagner S. 2009. New cherry cultivars obtained by mutagenesis at the fruit research station Cluj, Romania. Bulletin UASVM Horticulture 66(1): 215-217.

Rimieri P. 2017. La diversidad genética y la variabilidad genética: dos conceptos diferentes asociados al germoplasma y al mejoramiento genético vegetal. BAG. Journal of basic and applied genetics 28(2): 7-13.

Sahariya K, Kaushik RA, Khan R and Sarolia D. 2017. Influence of Gamma Irradiation on Flowering of Gladiolus (*Gladiolus hybrida* L.).

International Journal of Current Microbiology and Applied Sciences 6(11): 1362-1368. doi: 10.20546/ijcmas.2017.611.162

Satpute RA and Fultambkar RV. 2012. Mutagenic effectiveness and efficiency of gamma rays and EMS in soybean (*Glycine max* (L.) Merrill). Current Botany 3(2): 18-20

Silvera G. 2017. Mutaciones mediante radiaciones gamma en arroz. Revista Observatorio de la Economía Latinoamericana 235:1-16.

Singh S and Singh S. 1993. Effect of gamma rays on callus growth and plant regeneration in sugarcane CV Co 687. Indian Sugar 43(3): 181-182.

Singh A. 2007. Mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate in mungbean. Madras Agricultural Journal 94(1-6): 7-13.

Sonone NG, Deshmukh SN, Lanjewar SB and Bhakre RL. 2010. Induced variability in quantitative characters of groundnut (*Arachis hypogaea* L.). Electronic Journal of Plant Breeding 1(6): 1409-1414.

Subramanian A, Nirmalakumari A. and Veerabadhiran P. 2011. Mutagenic efficiency and effectiveness in kodomillet (*Paspalum scrobiculatum* L.). Madras Agricultural Journal 98 (1-3): 22-25.

Velmurugan M, Rajamani K, Paramaguru P, Gnanam R, Bapu K, Harisudan C and Hemalatha P. 2010. *In vitro* mutation in horticultural crops. Agricultural Reviews 31(1): 63-67.

Research article

http://www.revistas.unal.edu.co/index.php/refame

Revista Facultad Nacional de Agronomía

In situ assessment of *Jatropha curcas* germplasm under tropical dry forest conditions in Manabí-Ecuador

Evaluación *in situ* de germoplasma de *Jatropha curcas* bajo condiciones de bosque seco tropical en Manabí-Ecuador

UNIVERSIDAD NACIONAL DE COLOMBIA

doi: 10.15446/rfnam.v73n3.85788

Álvaro Cañadas-López[1]*, Diana Rade-Loor[2] and Carlos Molina-Hidrovo[3]

Keywords:
Biodiesel
Genetic gain
Genetic parameters
Heritability
Jatropha curcas

ABSTRACT

Jatropha germplasm accessions need an assessment of their traits to evaluate the nature and magnitude of the genetic variability among accessions. The objective of this research was to evaluate the phenotypic behavior of 130 *Jatropha curcas* L. (*Jatropha*) accessions and the genetic variability of selected *Jatropha* accessions. The selected variables were assessed between 2008-2014 based on the number of fruits per tree (FP), the number of seeds per fruit (SpF), weight of 100 dry seeds (100SW), seed length (SL), seed width (SW), smallest deviation standard as regular seed production (RP), highest deviation standard as irregular seed production (IP), seed oil content (Oil), seed production in g per tree (SP). Correspondence Analysis techniques were also applied in selected elite *Jatropha* accessions. The genotypic and phenotypic correlation coefficient between seed length, seed width, 100-seed weight and oil content for selected *Jatropha* accessions were applied. Variance, genotypic and phenotypic coefficients of variation, heritability (broad-sense) and genetic advance were calculated for several *Jatropha* phenotypic characteristics. CP041, CP052, CP037, CP054, CP060, CP122, CP118, CP120, CP121 INIAP *Jatropha* accessions were selected basically for SP and FP. A high statistically significant correlation (genotypic and phenotypic) between seed length – seed width was obtained from the chosen *Jatropha* accessions. Genetic association in the characteristics of growth and production highlighted the low phenotypic diversity in the *Jatropha* Portoviejo Research Station (EEP) of the National Institute for Agricultural and Cattle Ranching Research (INIAP) germplasm bank. There is an urgent need to improve the germplasm resource by obtaining new accessions, mainly from countries considered as centers of origin of the species.

Palabras clave:
Biocombustible
Ventaja genética
Parámetros genéticos
Heredabilidad
Jatropha curcas

RESUMEN

Los bancos de germoplasma de *Jatropha* necesitan una evaluación de sus rasgos para evaluar la naturaleza y la magnitud de la variabilidad genética entre accesiones. El objetivo de esta investigación fue evaluar el comportamiento fenotípico de 130 accesiones de *Jatropha curcas* L. (*Jatropha*) y luego analizar la variabilidad genética de las accesiones seleccionadas. La primera evaluación fue entre 2008-2014 con base en el número de frutos por árbol (FP), número de semillas por fruto (SpF), peso de 100 semillas secas (100SW), longitud de semilla (SL), ancho de semilla (SW), menor desviación estándar para la producción de semilla regular (RP), mayor desviación estándar para producción de semilla irregular (IP), contenido de aceite de semillas (Oil), producción de semilla en g por árbol (SP) y se aplicaron técnicas de análisis de correspondencia para la selección de las accesiones de *Jatropha* élite. Coeficiente de correlación genotípica-fenotípica entre longitud -ancho de semilla, peso de 100 semillas y contenido de aceite para accesiones seleccionadas de *Jatropha*. Se calculó la varianza, el coeficiente de variación genotípico y fenotípico, heredabilidad (sentido amplio) y ventaja genética para algunas características fenotípicas. Las accesiones INIAP CP041, CP052, CP037, CP054, CP060, CP122, CP118, CP120, CP121 fueron seleccionadas básicamente por su SP y FP. Se obtuvo una alta correlación estadísticamente significativa (genotípica y fenotípica) entre el ancho y longitud de la semilla de las accesiones de *Jatropha* seleccionadas. La asociación genética entre las características de crecimiento y producción resaltó la baja diversidad fenotípica del banco de germoplasma de *Jatropha* EEP- INIAP. Existe la urgente necesidad de mejorar el recurso de germoplasma mediante la obtención de nuevas accesiones principalmente, en países donde que se consideran centros de origen de la especie.

[1] Instituto Nacional de Investigaciones Agropecuarias - INIAP. Km 5 vía Quevedo—El Empalme, Cantón Mocache. CP. 120501, Provincia Los Ríos, Ecuador.
[2] Centro de Investigación de las Carreras de la ESPAM-MFL. Escuela Superior Politécnica de Manabí (ESPAM-MFL). Sitio El Limón, Calceta, Cantón Bolívar CP 130250. Provincia de Manabí, Ecuador.
[3] Instituto Nacional de Investigaciones Agropecuarias – INIAP. Av. Francisco Orellana y Justino Cornejo. CP. 170518, Guayaquil, Provincia del Guayas, Ecuador.
* Corresponding author: <alvarocaniadas@yahoo.es>

Received: April 16, 2020; Accepted: July 9, 2020
Rev. Fac. Nac. Agron. Medellín 73(3): 9273-9281. 2020

ISSN 0304-2847 / e-ISSN 2248-7026

Jatropha (Jatropha curcas L.) has not yet captured the necessary interest in its systematic improvement for better plantation yields (Montes *et al*., 2014). *Jatropha* enhanced varieties with desirable characteristics under suitable growing conditions are not available, which contributes to risk for the future of their sustainable production (Rao *et al*., 2017). *Jatropha* genetic resources could be enhanced through a global exploration, where the introduction of genetic materials, characterization and evaluation could provide essential information for the development of elite varieties through various methods (Morris *et al*., 2013). The key to a conventional breeding program lies in the availability of genetic variations of a specific desirable characteristic (Varshney *et al*., 2013). Rosado *et al*. (2010) found accessions from Asia, Africa and South America with low genetic diversity of *Jatropha*, while accessions with high genetic variability were found in Guatemala and Chiapas, Mexico.

Currently, elite *Jatropha* germplasm in the world is not available. Materials that combine several desirable traits are needed to establish large-scale plantations to ensure their economic viability (Singh *et al*., 2016). Therefore, there is almost no germplasm exchange. Only local materials are used, but these are not interchanged between regions (Rade-Loor *et al*., 2017). The growth of trees is determined by the influence of their genetic composition, the environment and the interaction between them. This interaction must be evaluated in breeding programs to measure plantation improvement in multiple environments. For breeders, it is vital to identify *Jatropha* materials, where this interaction does not occur. It ensures consistency among cultivar performances within a regional environment. Hence, selection based on a single environment will always be inferior to that based on multiple environments (Senger *et al*., 2016). According to Basha and Sujatha (2007), identification of promising lines within a germplasm bank of *Jatropha* may require a study covering typically a period of 5 to 10 years.

Several studies on *Jatropha* genetic diversity have been carried out to determine the genetic variation in populations (de Azevedo *et al*., 2017). In Ecuador, the National Institute of Agricultural Research (INIAP), within the Portoviejo Experimental Station (EEP), carried out an extensive national collection to establish a conservation and evaluation program for *Jatropha* accessions in 2008. The purpose of this germplasm bank was to test accessions, which included the analyses of genetic variability, broad-sense heritability and genetic advantage to establish clonal seed orchards to cover the *Jatropha* elite materials needed under the local tropical dry forest conditions (Cañadas-López *et al*., 2017). The information obtained will serve to form a collection of genotypes with desirable characteristics for future use in a genetic improvement program of this species for biodiesel production in Ecuador. The objectives of the present investigation were to evaluate the phenotypic behaviors of 130 *Jatropha* accessions and to characterize the accessions based on genetic variability of the selected accessions.

MATERIALS AND METHODS

The study was conducted at the Portoviejo Research Station (EEP), which belongs to the National Institute for Agricultural and Cattle Ranching Research (INIAP) (0°6'S, 80°23'W), Lodana Sector, Canton Portoviejo, Province of Manabí, Ecuador. The EEP is located at 47 masl, with a mean annual temperature of 26.3 °C, mean annual precipitation of 809.6 mm, mean relative humidity of 83% and mean number of sunshine hours of 1,159.3 h year^{-1} (Cañadas-López *et al*., 2018a; Cañadas-López *et al*., 2018b). The research area is ecologically classified as a tropical dry forest (Cañadas, 1983).

The collection of germplasm consists of 91% of materials from Ecuador (Figure 1), 5% from Brazil and 4% from Peru. After the establishment of the germplasm bank in 2008, the materials brought from abroad were not properly adapted to local conditions.

A total of 130 *Jatropha* accessions remained in the field. Plus-tree criteria were followed for selection according to Cornelius (1994). The selection was conducted in order to highlight phenotypic characteristics of interest such as the number of fruits per tree (FP), the number of seeds per fruit (SpF), weight of 100 dry seeds (100SW), seed length in cm (SL), seed width in cm (SW), lower standard deviations as regular seed production (RP), high standard deviation as an indicator of irregular seed production (IP), oil content of seeds in percentage (Oil) and seed production in g per tree (SP). The seeds of

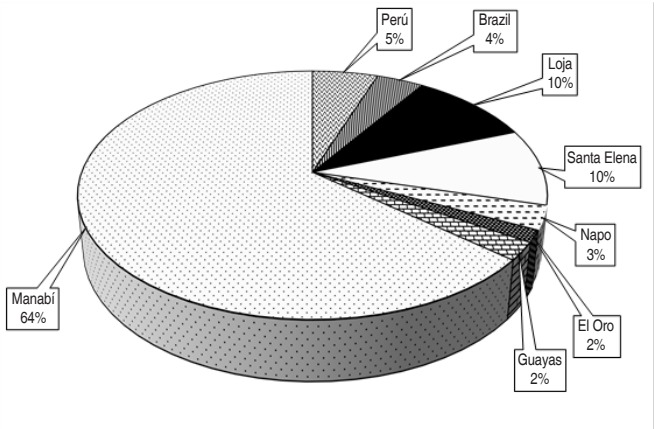


Figure 1. Origin of collected *Jatropha* accessions of the EEP-INIAP germplasm bank, Manabí.

all *Jatropha* accessions were separated, cleaned and stored in cotton bags under environmental conditions to establish seed characteristics. The seed lots were dried in a hot air oven at 60 °C until seeds reached a constant weight and a humidity of 8%. Five samples were taken from each seed batch, and 100 randomly selected undamaged seeds (total 500 were measured in length and width). The Soxhelt method was applied to estimate the oil content of 200 seeds with three replications for each seed lots (Cañadas-López *et al*., 2018). Once the collection was assembled, germplasm cuttings from plus-tree selection planted in 2008 with three replications totaling 12 plants per batch with a spacing of 2x2 m and a germplasm bank was established in EEP-INIAP.

In 2014, *Jatropha* germplasm was evaluated according to the above indicated variables. Techniques of correspondence analysis were applied. The analysis of cross-tabular data in form of numerical frequencies was particularly helpful since it shows an elegant but straightforward graphical display, which permits a rapid interpretation and understanding of the data (Bortz and Schuster, 2011). A primary indicator of analysis results was the inertia that can be interpreted as the weighted average of squared χ^2 distances between the average of the observed variables and the *Jatropha* accessions of different provenance regions. For this purpose, the software Statistica version 13.3 was applied.

During June 2015 and August 2016, *Jatropha* accessions described under the above criteria were sown in a system 2x2 m with three replications. Four *Jatropha* trees were selected from 18 month old, for each replication of each accession and data was recorded with the following morphological characteristics:

- Height of plants (cm): it was measured from soil level to the apex and results were reported as average.
- Number of branches per plant: The number of branches growing from the main stem in different positions of the nodes, including basal branches were counted.
- Number of bunches per plant: Expressed as average number of flower bunches per plant.
- Number of fruits per plant: The average number of fruits per plant.
- Number of seeds per fruit: Seed average found within the *Jatropha* fruit.
- Weight of 100 seeds: All batches of seeds were dried under similar temperatures and humidity conditions until reaching a constant weight. Three samples were taken from each batch of seeds and 100 were randomly selected to measure their weight.
- Seed yields: The weights of the seeds were established with a precision balance. After removing the pulp from the yellow fruits, the extracted seeds were dried in a convection oven at 60 °C for two days.

Analysis of variance and mean comparison were carried out with Statistica Software, version 13.3. Both genotypic and phenotypic correlation coefficients were calculated according to Johnson *et al*. (1955). Variability, genetic advance as a mean percentage, phenotypic and genotypic variance, phenotypic variation coefficient

(PCV) and genotypic variation coefficient (GCV) were calculated for seed oil content (Yoshida *et al*., 2007). Accession heritability was estimated by dividing the variance of the measurements into components between accessions and within accessions. For the genotypic variance, the difference between and within the selected accessions was calculated. In addition, the variance within accessions has been defined as phenotypic variance. Subsequently, the genotypic variance was divided by the phenotypic variance to obtain the broad-sense heritability. Genetic advance (%) was estimated as the difference between the genotypic mean of the accessions and the genotypic mean of the population.

RESULTS AND DISCUSSION

Phenotypic behavior of 130 Ecuadorian *Jatropha* accessions - evaluation 2008-2014

Figure 2 shows the first two dimensions from the correspondence analysis of the 130 *Jatropha* accessions and their nine variables. A very large proportion (96.2%) of the inertia is displayed along the first dimension. SP and FP were the main contributors to this dimension. These variables are opposed by a cluster of the variables SL, SW, SpF and 100SW. The second axis explains only 2.1% of the inertia. In this case, the main contributors were regular seed production (RP) and oil content (Oil). These were primarily opposed to IP and a lower extent, to the variables mentioned above that form a cluster. The accessions located close to the variables SP and FP were CP041, CP052, CP037, CP054, CP060, CP122, CP118, CP120, and CP121.

The highest production of seed and fruits from the 130 tested accessions of *Jatropha* are shown in Table 1, which are characteristics of 9 elite *Jatropha* accessions. The oil content, another important production factor, was opposite to the factors SP and FP. The values of the oil content of the nine highlighted accessions were indeed inconsistent and vary around the overall average of all tested accessions.

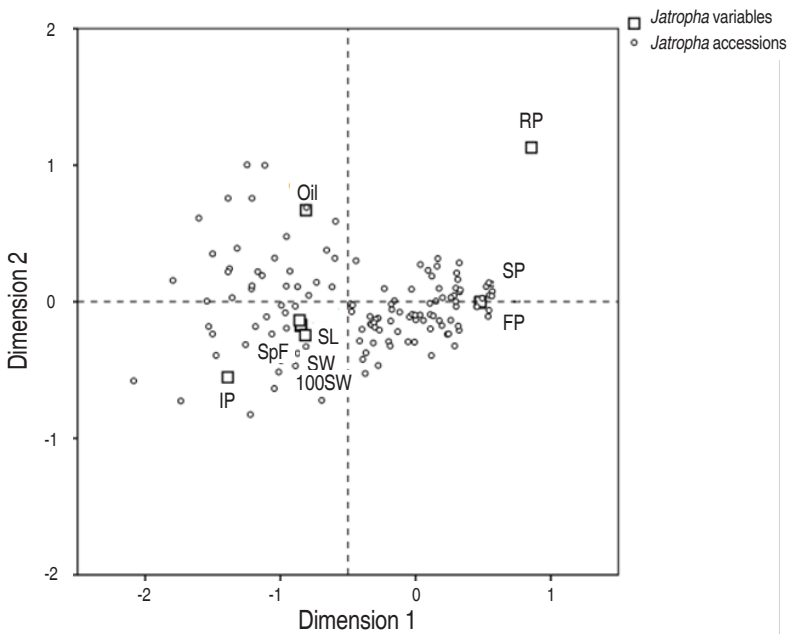


Figure 2. Dimensions 1 and 2 of the correspondence analyses of 130 *Jatropha* accessions, highlighting ten relevant variables. Number of fruits per tree (FP), number of seeds per fruit (SpF), 100-seed weight (100SW), seed length (SL), seed width (SW), regular seed production (RP), irregular seed production (IP), oil content (Oil) and seed production (SP).

In Ecuador, under tropical dry conditions, Cañadas-López *et al.* (2017) established for three *Jatropha* accessions a production of 0.70-0.75 t ha^{-1} year^{-1} for a 7-year-old plantation. The height of the *Jatropha* dry seed production variation was influenced by a factor unrelated to the water availability in the soil. At the same time, Cañadas-López *et al.* (2018) observed a production of 316.46 g tree^{-1} year^{-1} (791.15 t ha^{-1} year^{-1}) for the *Jatropha* accession CP041

Table 1. The *Jatropha* accessions with the highest seed production and the number of fruits, and summary statistics of 9 accessions and 130 accessions tested.

Elite *Jatropha* Accessions	Seed production (g)	Number of fruits	Seed length (mm)	Seed width (mm)	Seed number per fruit	100-seed weight (g)	Oil content (%)
CP041	730.73	147.97	18.28	10.96	2.80	78.60	51.51
CP052	652.26	110.60	18.38	10.88	2.50	75.66	46.77
CP037	588.33	138.16	18.41	11.51	2.80	80.70	31.95
CP054	404.89	132.78	17.35	10.90	2.80	79.15	50.01
CP060	356.10	119.57	19.30	11.25	2.80	68.00	24.94
CP122	180.54	85.97	17.33	10.94	2.67	69.70	43.95
CP118	180.43	85.92	17.77	11.13	2.80	73.40	36.63
CP120	159.12	75.77	18.24	11.21	2.80	77.50	34.03
CP121	157.96	75.22	18.36	11.32	2.90	74.80	36.68
130 access.							
Average	153.23	72.96	18.09	10.93	2.20	72.80	33.66
Maximum	730.73	347.97	20.60	12.45	3.00	87.77	55.81
Minimum	0.00	0.00	16.00	9.20	2.00	53.40	20.15
Standard error ±	12.31	0.58	0.07	0.04	0.01	0.58	0.62

of an eight-year-old. It would mean that *Jatropha*'s productivity could be lower than 1000 kg ha^{-1} $year^{-1}$ under dryland conditions in Ecuador (Cañadas-López *et al*., 2020). These productions registered in Ecuador were below the production variability ranges reported by Openshaw (2000) between 0.4 to 12 t ha^{-1} $year^{-1}$. Thus, *Jatropha* breeding programs are an important task in improving the dry seed production on marginal lands.

Yong *et al*. (2010) under the effects of four fertilizers in Sigapour observed an average number of fruits per plant of 81 (240 g (T240) of Osmocote® Plus). Joshi *et al*. (2011) registered 33 fruits per tree and a fruit yield per plant of 27 in 2009. These trees were sprayed with Ethrel (growth regulator) 150 ppm in the year 2008. Mohapatra and Panda (2010) registered for 18 *Jatropha* accession from India, an average of 77 fruits per tree. Comparable results were observed for the 130 Ecuadorian *Jatropha* accessions under dry forest conditions with an average of 75 fruits per tree.

Jatropha has a three-locular ovary and generally the fruit has three seeds (Divakara *et al*., 2010). In some Mexican *Jatropha* genotypes, four seeds per fruit are found under normal growth conditions (Makkar and Becker, 2009). For *Jatropha* accessions coming from six different countries, Nietsche *et al*. (2014) reported ranges between 2.63 to 2.96 seeds per fruit. In the present research, the number of seeds per fruit was established with an average of 2.20 seeds per tree and was lower to the other authors.

The average of 130 *Jatropha* accessions was 36.68% (±0.62) of oil content for the *Jatropha* accessions in Ecuador under dry forest conditions. Carels (2009) mentioned that 35% of oil seed content could be seen as a good *Jatropha* accession. Superior oil contents were found by Martínez-Díaz *et al*. (2017) with a *Jatropha* oil seed variation from 42.35% to 55.39% for different Mexican *Jatropha* populations. In Colombia, the percentage of *Jatropha* oil seed content varied between 37.2% and 40.1% (Montenegro *et al*., 2014).

Ginwal *et al*. (2005) established that in India the 100-seed weights were highly variable and these were under strong genetic control in comparison to environmental influence. Wani *et al*. (2012) found the 100-seed weight between 49 to 69 g in the Andhra Pradesh State, India. While for Malaysian *Jatropha* accessions, Shabanimofrad *et al*. (2013) registered weights from 44 to 77 g. The 100-seed weight of this study was 74.49 g, which is in the range of the other reports.

Nietsche *et al*. (2014) reported a seed length of 18.43 mm and 18.56 mm seed width for 15 *Jatropha* accessions and argued that a lower availability of water periods influences in seed characteristics such as seed shape. Under dry conditions, the cell turgor pressure is influenced to increase

cell expansion and carbohydrate accumulation. In the present investigation, it was possible to observe greater lengths (18.93 mm) and narrower widths (10.93 mm) of *Jatropha* seeds, although the seed ellipsoid shape prevailed.

Genetic association in growth and production characteristics of nine elite *Jatropha* selected accessions, evaluations in 2015 and 2016

There is a strong correlation (genotypic and phenotypic) between seed length and width. In contrast, there is no significant relationship (genotypic and phenotypic) between the seed width and 100-seed weight, nor between 100-seed weight and oil content (Table 2). The morphology characterization of seeds from Ecuadorian *Jatropha* accessions at EEP is the first step to determine the population's genetic variability. However, biotic and abiotic factors influence on the seed size (Valdés-Rodríguez *et al*., 2018).

The degree of correlation coefficient at genotype level was greater than its corresponding phenotypic coefficient correlations for seed width and seed length parameters (Table 2). This trend was congruent with those obtained by Valdés-Rodríguez *et al*. (2018) in Mexico. Nevertheless, the oil seed content was not related to any other variables analyzed. These results were in contrast with those found by Rao *et al*. (2008), who argued that the seed weight could be considered as an important trait for the early selection of seed sources.

Table 2. The correlation coefficient between seed length, seed width, 100-seed weight and oil content for nine selected *Jatropha* accessions, EEP-INIAP, Manabí-Ecuador.

Characteristics	Genotypic/ Phenotypic	Seed length (mm)	Seed width (mm)	100-seed weight (g)
Seed width (mm)	Genotypic	0.398 ***		
	Phenotypic	0.354 ***		
100-seed weight (g)	Genotypic	0.267 ns	0.282 ns	
	Phenotypic	0.285 ns	0.292 ns	
Oil content (%)	Genotypic	0.195 ns	0.195 ns	0.259 ns
	Phenotypic	0.197 ns	0.197 ns	0.260 ns

*** $P<0.001$; ns: not significant statistically

Genetic association in the characteristics of growth and production of nine elite *Jatropha* selected accessions

The maximum variation was observed for the *Jatropha* tree height followed by dry seed production and the number of fruit tree^{-1} (Table 3). At the same time, the minimum variance was detected in seed length, seed width, the weight of 100-seed weight, oil content, and the number of seeds per tree. The phenotypic variation coefficient was similar to the genotypic variation coefficient. The lowest genotypic variance was recorded for the seed number fruit^{-1} (8.41-10.30) and seed weight (9.81-13.87). Broad-sense heritability of seed length was 88%, 100-seed weight was 86% and of oil content 80% (Table 3). The genetic advance ranged from 3.81% (oil content) to 19.09% (seed length) for the nine tested *Jatropha* accessions.

A close correspondence was observed between the genotypic and phenotypic variance coefficient for all *Jatropha* accessions studied. Since the variation depends on the unit's magnitude measuring of the traits, the coefficient of variation is independent of its unit of measure. It is useful for comparing populations (Martin and Montes, 2015). For dry seed production, tree height and the FP of the Ecuadorian *Jatropha* accessions, a high genotypic and phenotypic coefficient of variation was found. These data can be compared with the results obtained by Rao *et al*. (2008) for production (54.90-55.26) and tree height (26.71-28.43). This fact indicates that the selection can be applied to the traits to isolate a more promising line. The same results were reported by Martin and Montes (2015). Nevertheless, the high PCV and GCV for the studied morphological characters of the *Jatropha* tree indicated that environmental influences on the expression of these traits were minor (Tefera *et al*., 2003).

Table 3. Genetic variable estimations for seed and oil traits in INIAP *Jatropha* accessions, EEP-INIAP, Manabí-Ecuador.

Variables	Range	Variables		Range		Heritability (broad-sense) %	Genetic advance
		Genotypic	Phenotypic	Genotypic	Phenotypic		
SP	153.23-730.73	93.3	135	53.63	64.86	68	4.19
FP	72.96-147.97	25.8	36.77	48.94	59.73	70	7.11
SL	1.11-2.06	0.07	0.08	19	20.31	88	19.09
SW	0.92-1.19	0.01	0.02	9.81	13.87	50	13.46
SpF	2.50-2.90	0.02	0.03	8.41	10.30	67	7
100SW	64.43-84.85	2.3	2.67	17.51	18.87	86	2.83
Oil	20.13-56.32	0.87	1.09	15.92	17.82	80	3.81
Tree height	165.04-300.00	113.3	161.52	76.93	91.86	70	7.78

SP: seed production; FP: fruits per tree; SL: seed length; SW: seed width; SpF: seed per fruit; 100SW: 100-dried seeds.

Seed length, 100-seed weight and oil content showed a moderate coefficient of genetic and phenotypic variability. According to Patil (2010), this reasonable variation could be improved by exhaustive selection. The characteristics of seed length and 100-seed weight displayed a low coefficient of variance, indicating the need of other resources with high variability of these characteristics.

However, characters that exhibit maximum heritability and high genetic advances as a mean could be used as a powerful selection tool. Such traits are controlled by the additive genes and are less influenced by the environment (Ahsan *et al*., 2015). For all *Jatropha* characteristics, a high broad-sense heritability was observed in the present study. In observed populations, the variation is due to both genetic and environmental factors, because genetic variability is the only inheritable from generation to generation. Heritability alone does not give a clear idea about the expected genetic gain in the succeeding generation and therefore, it should be considered as a whole with the genetic advances (Alves *et al*., 2013).

Hence, the combination of high broad-sense heritability and high genetic advantage will provide a clear basis on the reliability of that particular trait in the selection of *Jatropha* varieties (Hernández-Velasco *et al*., 2016; Ortiz-Olivas *et al*., 2017) It was not observed in the present investigation on the elite EEP-INIAP *Jatropha* accessions.

CONCLUSION

In an environment of global warming and food security problems due to human population growth, priority should be given to plants that are not edible, but that can be used for the production for biodiesel. *Jatropha*'s potential as an organic biodiesel raw material in promoting socio-economic development and meeting energy demands as the "*Jatropha* for Galápagos" Project does, which needs superior *Jatropha* materials. However, the results of this study highlight the low phenotypic diversity of the physically dry seed variability within the *Jatropha* EEP-INIAP germplasm bank under tropical dry forest conditions. It could be because 72% of the material collected comes from the Manabí Province. The *Jatropha* vegetative propagation form, mostly diffused in Manabí, is by stakes, which could have caused the lack of variability, reflecting a common descendant. There is an urgent need to improve the germplasm resource by obtaining new accessions, mainly from countries considered as centers of origin of the species to promote the genetic diversity necessary to develop a genetic improvement program for the *Jatropha* plantations.

ACKNOWLEDGMENTS

The authors thank the Director of the Portoviejo Experimental Research Station (EEP) of the National Institute of Agricultural Research (INIAP) period 2015–2016 and INIAP General Director for the *Jatropha* data release and the updating of the information. They also thank Michael Anthony McLaughlin and Marcelino de Souza Lima for the editing and review.

REFERENCES

Ahsan MZ, Majidano MS, Bhutto H, Soomro AW, Panhwar FH, Channa AR and Sial KB. 2015. Genetic variability, coefficient of variance, heritability and genetic advance of some *Gossypium hirsutum*

L. accessions. Journal of Agricultural Science 7(2): 147. doi: 10.5539/jas.v7n2p147

Alves MF, Barroso PAV, Ciampi AY, Hoffmann LV, Azevedo VCR and Cavalcante U. 2013. Diversity and genetic structure among subpopulations of *Gossypium mustelinum* (Malvaceae). Genetics and Molecular Research 12(1): 597-609. doi: 10.4238/2013.February.27.9

Basha SD and Sujatha M. 2007. Inter and intra-population variability of *Jatropha curcas* (L.) characterized by RAPD and ISSR markers and development of population-specific SCAR markers. Euphytica 156(3): 375-386. doi: 10.1007/s10681-007-9387-5

Bortz J and Schuster C. 2011. Statistik für Human-und Sozialwissenschaftler: Limitierte Sonderausgabe. Seventh edition. Springer-Verlag. Berlin, Heildelberg, New York. pp. 80-120. [In German].

Cañadas-López Á, Rade-Loor D, Siegmund-Schultze M, Zambrano-Cedeño X, Vargas-Hernández J and Wehenkel C. 2020. Pruning and fertilization effects on *Jatropha* yields under smallholder's conditions in a Tropical Dry Forest from Ecuador. Revista Facultad Nacional de Agronomía Medellín 73(1): 9089-9097. doi: 10.15446/rfnam.v73n1.79059

Cañadas-López Á, Andrade-Candell J, Domínguez-Andrade, JM, Molina-Hidrovo C, Schnabel-Delgado O, Vargas-Hernández JJ and Wehenkel C. 2018a. Growth and yield models for teak planted as living fences in coastal Ecuador. Forests 9(2): 55. doi: 10.3390/f9020055

Cañadas-López Á, Rade-Loor D, Siegmund-Schultze M, Iriarte-Vera M, Domínguez JM, Vargas-Hernández J and Wehenkel C. 2018b. Productivity and Oil Content in Relation to *Jatropha* Fruit Ripening under Tropical Dry-Forest Conditions. Forests 9(10): 611. doi: 10.3390/f9100611

Cañadas-López Á, Rade-Loor D, Domínguez-Andrade JM, Vargas-Hernández JJ, Molina-Hidrovo C, Macías-Loor C and Wehenkel C. 2017. Variation in seed production of *Jatropha curcas* L. accessions under tropical dry forest conditions in Ecuador. New Forests 48(6): 785-799. doi: 10.1007/s11056-017-9597-1.

Cañadas L. 1983. El mapa ecológico y bioclimático del Ecuador. Editores Asociados Cia. Ltd. Quito, Ecuador. pp. 50-120.

Carels N. 2009. *Jatropha curcas*: a review. Advances in Botanical Research 50: 39-86. doi: 10.1016/S0065-2296(08)00802-1

Cornelius J. 1994. The effectiveness of plus-tree selection for yield. Forest Ecology and Management 67(1-3): 23-34. doi: 10.1016/0378-1127(94)90004-3

de Azevedo Peixoto L, Laviola BG, Alves AA, Rosado TB and Bhering LL. 2017. Breeding *Jatropha curcas* by genomic selection: a pilot assessment of the accuracy of predictive models. PLoS One 12: 0173368. doi: 10.1371/journal.pone.0173368

Divakara BN, Upadhyaya HD, Wani SP and Gowda CL. 2010. Biology and genetic improvement of *Jatropha curcas* L.: a review. Applied Energy 87(3): 732-742. doi: 10.1016/j.apenergy.2009.07.013

Ginwal HS, Phartyal SS, Rawat PS and Srivastava RL. 2005. Seed source variation in morphology, germination and seedling growth of *Jatropha curcas* Linn. in central India. Silvae Genetica 54(1-6): 76-80. doi: 10.1515/sg-2005-0012

Hernández-Velasco J, Hernández-Díaz JC, Fladung M, Cañadas-López Á, Prieto-Ruíz and JÁ, Wehenkel, C. 2017. Spatial genetic structure in four *Pinus* species in the Sierra Madre Occidental, Durango, Mexico. Canadian Journal of Forest Research 47(1): 73-80. doi: 10.1139/cjfr-2016-0154

Johnson HW, Robinson HF and Comstock RE. 1955. Genotypic and phenotypic correlations in soybeans and their implications in selection. Agronomy Journal 47(10): 477-483. doi: 10.2134/agronj1955.00021962004700100008x

Joshi G, Shukla A and Shukla A. 2011. Synergistic response of auxin and ethylene on physiology of *Jatropha curcas* L. Brazilian Journal of Plant Physiology 23(1): 66-77. doi: 10.1590/S1677-04202011000100009

Makkar HP and Becker K. 2009. *Jatropha curcas*, a promising crop for the generation of biodiesel and value-added coproducts. European Journal of Lipid Science and Technology 111(8): 773-787. doi: 10.1002/ejlt.200800244

Martínez-Díaz Y, González-Rodríguez A, Rico-Ponce HR, Rocha-Ramírez V, Ovando-Medina I and Espinosa-García FJ. 2017. Fatty acid diversity is not associated with neutral genetic diversity in native populations of the biodiesel plant *Jatropha curcas* L. Chemistry & Biodiversity 14(1): e1600188. doi: 10.1002/cbdv.201600188

Martin M and Montes JM. 2015. Quantitative genetic parameters of agronomic and quality traits in a global germplasm collection reveal excellent breeding perspectives for *Jatropha curcas* L. GCB Bioenergy 7(6): 1335-1343. doi: 10.1111/gcbb.12227

Mohapatra S and Panda PK. 2010. Genetic variability on growth, phenological and seed characteristics of *Jatropha curcas* L. Notulae Scientia Biologicae 2(2): 127-132. doi: 10.15835/nsb224592

Montenegro R, Magnitskiy S, Henao T and Martha C. 2014. Effect of nitrogen and potassium fertilization on the production and quality of oil in *Jatropha curcas* L. under the dry and warm climate conditions of Colombia. Agronomía Colombiana 32(2): 255-265. doi: 10.15446/agron.colomb.v32n2.43265

Montes JM, Technow F, Martin M and Becker K. 2014. Genetic diversity in *Jatropha curcas* L. assessed with SSR and SNP markers. Diversity 6 (3): 551-566. doi: 10.3390/d6030551

Morris GP, Ramu P, Deshpande SP, Hash CT, Shah T, Upadhyaya HD, Riera-Lizarazy O, Brown P, Acharya ChB, Mitchell ShE, Harriman J, Glaubitz J, Bukler ES and Kresovich S. 2013. Population genomic and genome-wide association studies of agroclimatic traits in sorghum. Proceedings of the National Academy of Science 110(2): 453-458. doi: 10.1073/pnas.1215985110

Nietsche S, Vendrame WA, Crane JH and Pereira MCT. 2014. Assessment of reproductive characteristics of *Jatropha curcas* L. in south Florida. GCB Bioenergy 6(4): 351-359. doi: 10.1111/gcbb.12051

Openshaw K. 2000. A review of *Jatropha curcas*: an oil plant of unfulfilled promise. Biomass and Bioenergy 19(1): 1-15. doi: 10.1016/S0961-9534(00)00019-2

Ortiz-Olivas ME, Hernández-Díaz JC, Fladung M, Cañadas-López Á, Prieto-Ruíz, JÁ and Wehenkel, C. 2017. Spatial Genetic Structure within and among Seed Stands of Pinus engelmannii Carr. and Pinus leiophylla Schiede ex Schltdl. & Cham, in Durango, Mexico. Forests 8(1): 22. doi:10.3390/f8010022

Patil HE. 2010. Variability and correlation analysis by using various quantitative traits in released Bt cotton hybrids. Journal of Cotton Research and Development 24(2):141-144.

Rao GR, Korwar GR, Shanker AK and Ramakrishna YS. 2008. Genetic associations, variability and diversity in seed characters, growth, reproductive phenology and yield in *Jatropha curcas* (L.) accessions. Trees 22(5): 697-709. doi: 10.1007/s00468-008-0229-4

Rao GR, Prabhakar M, Sathi Reddy P and Sharath Kumar P. 2017. *Jatropha curcas* (L.) seed yields as impacted by pruning intensity and intercropping. Biofuels 8(3): 383-390. doi:

10.1080/17597269.2016.1231958

Rade-Loor D, Cañadas-López Á and Zambrano C. 2017. Silvopastoral system economical and financial feasibility with *Jatropha curcas* L. in Manabí, Ecuador. MVZ Cordoba 22(3): 6241-6255. doi: 10.21897/rmvz.1129

Rosado TB, Laviola BG, Faria DA, Pappas MR, Bhering LL, Quirino B and Grattapaglia D. 2010. Molecular markers reveal limited genetic diversity in a large germplasm collection of the biofuel crop *Jatropha curcas* L. in Brazil. Crop Science 50(6): 2372-2382. doi: 10.2135/cropsci2010.02.0112

Singh S, Prakash A, Chakraborty NR, Wheeler C, Agarwal PK and Ghosh A. 2016. Trait selection by path and principal component analysis in *Jatropha curcas* for enhanced oil yield. Industrial Crops and Products 86: 173-179. doi: 10.1016/j.indcrop.2016.03.047

Senger E, Martin M, Dongmeza E and Montes JM. 2016. Genetic variation and genotype by environment in *Jatropha curcas* L. germplasm evaluated in different environments of Cameroon. Biomass and Bioenergy 91: 10-16. doi: 10.1016/j.biombioe.2016.04.017

Shabanimofrad M, Rafii MY, Wahab PM, Biabani AR and Latif MA. 2013. Phenotypic, genotypic and genetic divergence found in 48 newly collected Malaysian accessions of *Jatropha curcas* L. Industrial Crops and Products 42: 543-551. doi: 10.1016/j.indcrop.2012.06.023

Tefera H, Assefa K, Hundera F, Kefyalew T and Teferra T. 2003. Heritability and genetic advance in recombinant inbred lines of tef (*Eragrostis tef*). Euphytica 131(1): 91-96. doi: 10.1023/A:1023009720870

Valdés-Rodríguez OA, Pérez-Vázquez A, Palacios-Wassenaar OM and Sánchez-Sánchez O. 2018. Seed diversity in Native Mexican *Jatropha curcas* L. and their environmental conditions. Tropical and Subtropical Agroecosystems 21(3): 521-537.

Varshney RK, Song C, Saxena RK, Azam S, Yu S, Sharpe AG, Cannon S, Baek J, Rosen BD, Tar'an B, Millan T, Zhang X, Ramsay LD, Iwata A, Wang Y, Nelson W, Farmer AD, Gaur PM, Soderlund C, Penmetsa RV, Xu Ch, Bharti AK, He W, Winter P, Zhao SH, Hane JK, Carrasquilla-Garcia N, Condie JA, Upadhyaya HD, Luo M-Ch, Thudi M, Gowda CLL, Singh NP, Lichtenzveig J, Gali KK, Rubio J, Nadarajan N, Dolezel J, Bansal KC, Xu X, Edward D, Zhang G, Kahl F, Gil J, Singh KB, Datta SW, Jackson SA, Wang J and Cook DR. 2013. Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. Nature Biotechnology 31(3): 240-246. doi: 10.1038/nbt.2491

Wani SP, Chander G, Sahrawat KL, Rao CS, Raghvendra G, Susanna P and Pavani M. 2012. Carbon sequestration and land rehabilitation through *Jatropha curcas* (L.) plantation in degraded lands. Agriculture, Ecosystems & Environment 161:112-120. doi: 10.1016/j.agee.2012.07.028

Yong JWH, Ng YF, Tan SN and Chew AYL. 2010. Effect of fertilizer application on photosynthesis and oil yield of *Jatropha curcas* L. Photosynthetica 48(2): 208-218. doi: 10.1007/s11099-010-0026-3

Yoshida H, Horie T, Katsura K and Shiraiwa T. 2007. A model explaining genotypic and environmental variation in leaf area development of rice based on biomass growth and leaf N accumulation. Field Crops Research 102(3): 228-238. doi: 10.1016/j.fcr.2007.04.006

Research article

http://www.revistas.unal.edu.co/index.php/refame

Revista Facultad Nacional de Agronomía

Gas exchange efficiency in Cocoa – Spanish elm agroforestry system in the northwest Antioquia, Colombia

Eficiencia del intercambio gaseoso en un sistema agroforestal de Cacao – Olmo español en el noroeste de Antioquia, Colombia

doi: 10.15446/rfnam.v73n3.85278

Juan Pablo Gómez-Yarce[1]*, Edna Rocío Mompotes-Largo[2], Aníbal López-Castro[1], Juan David Hernández-Arredondo[2] and Oscar de Jesús Córdoba-Gaona[1]

Keywords:
Cacao
Cordia alliodora
Net photosynthesis
Stomatal conductance
Theobroma cacao
Transpiration

ABSTRACT

The cultivation of cocoa (*Theobroma cacao* L.) under agroforestry systems, generates beneficial environmental conditions for cocoa crop physiology. An experiment was conducted to evaluate the effect of shade trees (Spanish elm trees - *Cordia alliodora* (Ruiz & Pavon) Oken) planted along with cocoa (clone CCN51) under an agroforestry system on cocoa's gas exchange parameters regarding the reduction of the light intensity over the cocoa-leaf canopy. The experiment was developed in the Centro de Investigación el Nus - Agrosavia, located in the municipality of San Roque, Antioquia. The experimental design used was a randomized complete block design for the cocoa planting distances from the first row of Spanish elm trees interfacing with the cocoa plantation (4 m, 7 m, 10 m, 13 m). The statistical analysis was performed by estimating the area under the curve (*AUC*) of each variable, using the trapezoid equation of the statistical environment SAS® 9.4, an analysis of variances was performed to determine if there were statistical differences between treatments, and Tukey's test at 5% probability was used to estimated statistical differences between means. There were significant differences in the treatments regarding the net photosynthetic rate (*A*), stomatal conductance (g_s), and transpiration rate (*E*). The highest values of gas exchange parameters were found in the plants located 13 m from elm trees, while the lowest values were presented at 4 m. Plants at 7 m and 10 m always showed intermediate values for all gas exchange parameters. In the same sense, plants at 13 m had a higher radiation use efficiency (*RUE*) compared to plants at 4 m. The arboreal component modified the environmental conditions on cocoa trees regarding its distribution, generating a differential response to the physiological behavior of cocoa plants.

Palabras clave:
Cacao
Cordia alliodora
Fotosíntesis neta
Conductividad estomática
Theobroma cacao
Transpiración

RESUMEN

El cultivo de cacao (*Theobroma cacao* L.) bajo sistemas agroforestales, genera condiciones ambientales beneficiosas para la fisiología de este cultivo. Se llevó a cabo un experimento para evaluar el efecto de los árboles de sombrío (árboles de olmo españoles - *Cordia alliodora)* plantados en asocio con cacao (clon CCN51) bajo un sistema agroforestal, para analizar los efectos de la intensidad de la luz que llega al dosel de la hoja de cacao sobre los parámetros de intercambio gaseoso. El experimento se desarrolló en el Centro de Investigación el Nus - Agrosavia, ubicado en el municipio de San Roque (Antioquia). El diseño experimental utilizado fue de bloques completos al azar para las distancias de plantación de cacao de la primera fila de olmos españoles en la intersección con la plantación de cacao (4 m, 7 m, 10 m, 13 m). El análisis estadístico se realizó estimando el área bajo curva de cada variable (*AUC*), utilizando la ecuación trapezoidal por medio del entorno estadístico SAS® 9,4, para determinar diferencias significativas entre tratamientos se realizó un análisis de varianza, y se usó la prueba de Tukey al 5% de probabilidad para estimar diferencias entre medias. Hubo diferencias en los tratamientos con respecto a la tasa neta de fotosintética (*A*), la conductividad estomática (g_s) y la transpiración (*E*). Los valores más altos de los parámetros de intercambio de gaseoso se encontraron en las plantas ubicadas a 13 m de los Olmos, mientras que los valores más bajos se presentaron en la posición de 4 m. Las plantas en posición 7 m y 10 m siempre presentaron valores intermedios para todos los parámetros de intercambio gaseoso. En el mismo sentido, las plantas en la posición de 13 m presentaron un mayor uso eficiente de la radiación (*RUE*) en comparación con las plantas en la posición de 4 m. El componente arbóreo modificó las condiciones ambientales sobre los árboles de cacao en relación con su distribución, generando una respuesta diferencial del comportamiento fisiológico de las plantas de cacao.

[1] Facultad de Ciencias Agrarias. Universidad Nacional de Colombia. AA 3840, Medellín, Colombia.
[2] Corporación Colombiana de Investigación Agropecuaria – AGROSAVIA. Centro de Investigación El Nus. Corregimiento de San José del Nus, San Roque, Antioquia, Colombia.
* Corresponding author: <jpgomezy@unal.edu.co>

Received: March 29, 2020; Accepted: July 16, 2020
Rev. Fac. Nac. Agron. Medellín 73(3): 9283-9291. 2020
ISSN 0304-2847 / e-ISSN 2248-7026

Cocoa (*Theobroma cacao* L.) is considered a perennial tropical species of high commercial value (Carr and Lockwood, 2011) because the seeds of the *Theobroma* genus are destined in the elaboration of chocolates and derived products that have high demand in the global market (Jaimez *et al*., 2008). The world cocoa production is 5.2 million t (FAOSTAT, 2017), where Ivory Coast is the world's largest producer with total estimated production with 1.98 million t (38%) of total grain yield production, followed by Ghana with 20%, and Cameroon with 6%. Colombia occupies 10th place with 1% of the production (ICCO, 2018).

Cocoa is cultivated in diverse environments, from humid to dry climates (García *et al*., 2005). It is considered a shade-tolerant species; thus, the crop is traditionally sown in multi-strata agroforestry systems, where cocoa trees are planted with fruit, timber, and non-timber species of high commercial value (Almeida and Valle, 2007; Niether *et al*., 2018). Wessel (1985) reported that shade conditions benefit the crop physiology of cocoa in these systems by decreasing irradiance, temperature, and airspeed flow, factors that influence the fixation of CO_2 and processes associated with the loss of water (Klich, 2000). Thus, the agroforestry system improves environmental conditions for cocoa cultivation, considered beneficial for the cocoa plant survival, because it is a plant profoundly affected by environmental stress conditions (Almeida and Valle, 2007). In contrast, Suárez Salazar *et al*. (2018) showed that cocoa plants exhibit optimal acclimatization with relatively high solar radiation by improving photosynthetic performance, contradicting the assumption that cocoa plants grow better under shade conditions.

In this sense, irradiance has great relevance because it is the main factor to regulate the assimilation rate of carbon dioxide in plants (Jaimez *et al*., 2018). Several authors report photosynthesis rates (*A*) between 3 and 8 µmol CO_2 m^{-2} s^{-1} and stomatal conductance (g_s) between 50 and 170 mmol H_2O m^{-2} s^{-1} (Baligar *et al*., 2008; Daymond *et al*., 2011; Araque *et al*., 2012; Acheampong *et al*., 2013). However, these values are considered relatively low compared with other species of the *Theobroma* genus, including *T. grandiflorum* and *T. subincanum* (Almeida *et al*., 2014). There is evidence that the photosynthetic rate of cocoa may increase if the photosynthetically active radiation (*PAR*) increases as well (Do Costa *et al*., 2001; Suárez Salazar *et al*., 2018). This increase is influenced not only by the type of tree shade species associated but by the time of day and year.

The shade tree is a significant factor to consider in the design and planting of agroforestry systems with cocoa (Galyuon *et al*., 1996). Cocoa is a crop with shade plants characteristics and that it requires this condition for better production because the cocoa plant has a low light saturation, with 95% of the maximum photosynthesis between 200 and 600 µmol CO_2 m^{-2} s^{-1} (Almeida *et al*., 2014). According to Tezara *et al*. (2016), cocoa's *A* is saturated with 400 µmol m^{-2} s^{-1} of photosynthetic photonic flux density (PPFD), considering the relationship between them (*A*/PPFD). Therefore, studies conducted by Acheampong *et al*. (2013) reported an increase in stomatal conductance in cocoa plants when they were subjected to a shade level of 75% (0.15 mol CO_2 m^{-2} s^{-1}). In comparison with plants exposed to higher irradiance (55 and 32% of shade in the dry season), the conductance reached values of 0.1 mol CO_2 m^{-2} s^{-1} and 0.09 mol CO_2 m^{-2} s^{-1}, respectively, which was due to lower temperature inside the system (3 °C). In terms of photosynthesis, the highest values were observed in plants with 55% of shade environment (0.82 µmol CO_2 m^{-2} s^{-1}), concerning those planted with 32% in shade condition (0.52 µmol CO_2 m^{-2} s^{-1}), where the highest photosynthetic rates occurred in the morning. It was hypothesized that the different mean levels of incident *PAR* on cocoa trees, according to their position and orientation (east-west) within an agroforestry system, affects the physiological performance of the gas exchange in this species. Therefore, this work aimed to evaluate the effect of shade trees planted along with *Theobroma cacao* L. on the gas exchange parameters regarding the reduction of the light intensity over the cocoa-leaf canopy.

MATERIAL AND METHODS

In September 2018, a field experiment was conducted at Centro de Investigación El Nus, Corporación Colombiana de Investigación Agropecuaria - AGROSAVIA (06° 26'17,2" N, 74° 49'32,1" W, 850 m.a.s.l.) in the municipality of San Roque, Antioquia, Colombia. Using

a portable weather station system "Wachtdog™ 2000", the annual average temperature (24 °C), average relative humidity (87%), and annual total rainfall (2500 mm), with two rainy seasons (from March to June and, September to November), and a dry season of low rainfall (December–February) were measured.

Randomized complete block design and six repetitions with four treatments were performed. Irradiance levels are given by the distance from the edge of the trees, in a cocoa agroforestry system, where the cocoa and shade trees, were five-years-old. Cocoa trees clone CCN51 (Colección Castro Naranjal) were planted with a distance of 3 m×3 m. The woody species associated with cocoa trees were Spanish elm (*Cordia alliodora* (Ruiz & Pavon) Oken), which belongs to the Boraginaceae family, established in double rows 16 m and, the trees, being spaced at shorter distances (3 m×3 m). The four treatments included distances measured from the first row of Spanish elm trees interfacing with the cocoa plantation (zero distance). The treatments evaluated were the distances of 4 m (A), 7 m (B), 10 m (C), and 13 m (D) from the west to the east edge of the Spanish elm trees (Figure 1). The position of cocoa trees within agroforestry arrangements was considered relevant since the availability of radiation is influenced by the movement of the sun from east to west; that is, position 4 m receives more radiation in the morning, while position 13 m, on the contrary, receives more radiation in the afternoon.

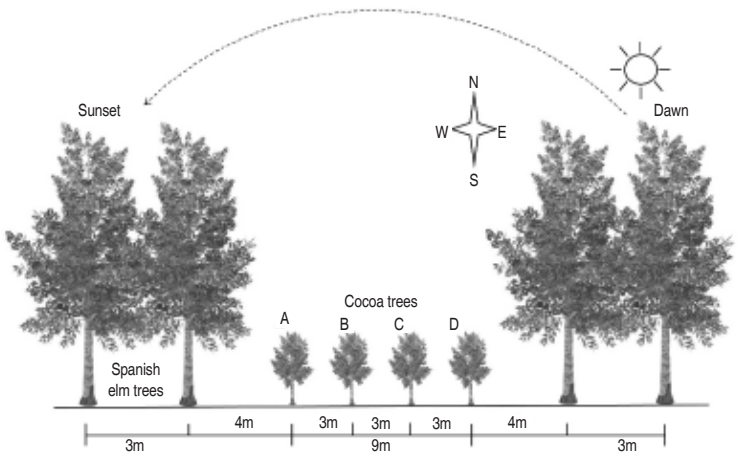


Figure 1. Cross-section of the experimental field showing the arrangement of Spanish elm (Nogal) trees and cocoa plants.

Light condition above cocoa plant canopies was continuously measured by SS1 SunScan Canopy Analysis System (Delta-T Devices Ltd), allowing estimating light transmission and availability to cocoa plants. Incident radiation (Ir) flux above the canopy and radiation flux transmitted (Tr) below the shade tree canopy was recorded every half hour, from 7:00 to 17:00 hours. The Spanish elm tree regimes shade (S) on cocoa plants were calculated by the equation S (%)=(1-(Tr/Ir)×100).

A portable photosynthesis-measuring system with infrared gas analyzer incorporated (LCi - ADC Bioscience, UK) was used to measure leaf gas exchange. Measurements were made on the third fully expanded leaf of the last mature shoot from the apex for five consecutive days in six cocoa plants (CCN51) for each of the positions within the agroforestry system. Net photosynthetic rate (*A*, μmol CO_2 m^{-2} s^{-1}), transpiration rate (*E*, mmol H_2O m^{-2} s^{-1}), stomatal conductance to water vapor (g_s, mol H_2O m^{-2} s^{-1}), and photosynthetically active radiation (*PAR*, μmol photons m^{-2} s^{-1}) were estimated from 8:00 to 17:00 hours. The instantaneous water use efficiency (*WUE*, mmol CO_2 $mmol^{-1}$ H_2O) and radiation use efficiency (*RUE*, mmol CO_2 μmol photons) was calculated by *A*/*E* and *A*/*PAR*, respectively.

Vapor pressure deficit (*VPD*, measured in kPa) was calculated from daily temperature and relative humidity recordings according to equation 1 proposed for Rosenberg

et al. (1983). The temperature (T) and humidity (RH) values were taken with the thermo-hygrometer (Thermo Hygro and Clock) at every half an hour interval during the day (7:00 to 17:00).

$$VPD=0.61078[(17.269xT/T+237.3)x(1+(RH/100)] \quad (1)$$

Statistical analysis

The area under the curve (*AUC*) was estimated to determine the accumulated value throughout the day of each physiological variable *A*, g_s, *E* and *PAR*. The *AUC* was estimated by fractioning the total in trapezoidal areas. These individual areas were calculated using the trapezoid equation of a macro of the statistical environment SAS® 9.4 developed by Córdoba-Gaona *et al.* (2018), which was adapted from those of the routines detailed by Huang and Xiao (2010) and Shiang (2004). *AUC* data were subjected to an analysis of variance. The differences among the means were determined with Tukey's test at 5% probability. The "agricolae" package (De Mendiburu, 2013) included in the R project statistical environment software was used (R Core Team, 2017).

RESULTS AND DISCUSSION

Diurnal gas exchange

Leaf temperature, environmental temperature, and the diurnal variation of gas exchange parameters in cocoa plant growth in several positions are presented in Figure 2. During the time of higher photosynthetic activity, the maximum net photosynthetic rate (*A*, 4.73 µmol CO_2 m^{-2} s^{-1}) was observed at 13:00 hours in the cocoa tree planted at 13 m from the west edge of Spanish elm. In contrast, the minimum photosynthesis corresponded to the cocoa plant at 4 m from the western edge of Spanish elm (0.86 µmol CO_2 m^{-2} s^{-1}) at 11:00 hours. From 13:00 to 17:00, the photosynthesis rate gradually decreases until minus zero values, indicating a negative *A* (Figure 2A). The lower photosynthetic activity in cocoa plants at 4 m from the eastern side of Spanish-helm trees, between 8:00 and 11:00 hours, is associated with the radiation incident on this position, reaching *PAR* values of 1,500 µmol photons m^{-2} s^{-1}, radiation above the saturation point for cocoa; contrary to the incident radiation at 13 m, which receives maximum values of 406 µmol photons m^{-2} s^{-1} between 8:00 and 10:00 hours (Figure 3A). Balasimha *et al.* (1991) have been reported that cocoa plants saturate at photonic flux densities between 400 and 600 µmol photons m^{-2} s^{-1}, which represents between 25 and 30% of the maximum radiation on a clear day, the maximum CO_2 assimilation rates do not exceed 6 to 7 µmol photons m^{-2} s^{-1}. It agrees with Niether *et al.* (2018), who indicated that the reduction of radiation generated by forest trees improves environmental conditions for cocoa cultivation because cocoa has a low light saturation point, where 95% of maximum photosynthesis occurs with a *PAR* of 200 µmol m^{-2} s^{-1} (Baligar *et al.*, 2008).

Similar to the observation in net photosynthetic rate, there was an influence on stomatal conductance and transpiration rate according to cocoa plants at different distances (Figures 2B and 2C). At 13 m, cocoa plant registered the highest g_s and *E* (0.152 mol H_2O m^{-2} s^{-1}, 5.89 mmol H_2O m^{-2} s^{-1}); in contrast, the lowest g_s and *E* were observed in the cocoa tree at 4 m (0.033 mol H_2O m^{-2} s^{-1}, 1.74 mmol H_2O m^{-2} s^{-1}). In all positions, stomatal conductance and transpiration rate presented a decrease starting from 14:00 to 17:00 hours. The high values of *VPD* constitute one of the main limiting factors for photosynthesis because of a reduction of *A* and a photorespiration increase. This behavior could probably be due to the effects of *VPD* on the closure of the stomata, which leads to a reduction of internal CO_2 (Almeida *et al.*, 2014). Regarding the variation of the water deficit pressure (*VPD*), the higher value was reported between 11:30 and 12:30 hours, values ranging from 0.645 to 0.643 kPa, respectively (Figure 2D).

The VPD is considered one of the main factors in stomatal opening and closing (Dos Santos *et al.*, 2017). An increase in the *VPD* was observed after 10:00 hours, where a direct relationship for all distances (4 m, 7 m, 10 m, 13 m) was found with *A*, g_s, and *E*. The maximum *VPD* (0.645 kPa) was recorded at 11:30 hours. This variation is due to the high temperatures of the environment (29.7 °C) and the low relative humidity (43%). This condition of *VPD* (Figure 2F), according to Ribeiro *et al.* (2009), induces the stomatal closure and generates a reduction in transpiration rate. Contrary to the present work, where *A*, *gs*, and *E* are proportional to *VPD*. As the *VPD* decreases, these gas exchange variables decrease and vice versa, although according to Köhler *et al.* (2014), cocoa species is sensitive to high values of pressure deficit. Therefore, a reduction in the

assimilation of CO_2 can be observed. This variable must increase above 2 kPa due to the *VPD* (Balasimha *et al*., 1991), which was never reached in this experiment, observing maximum values of 0.645 kPa. In general, the photosynthetic rates varied throughout the day, being higher during the morning compared to the afternoon in all cocoa plant positions. This behavior was associated with higher *VPD* values in the afternoon.

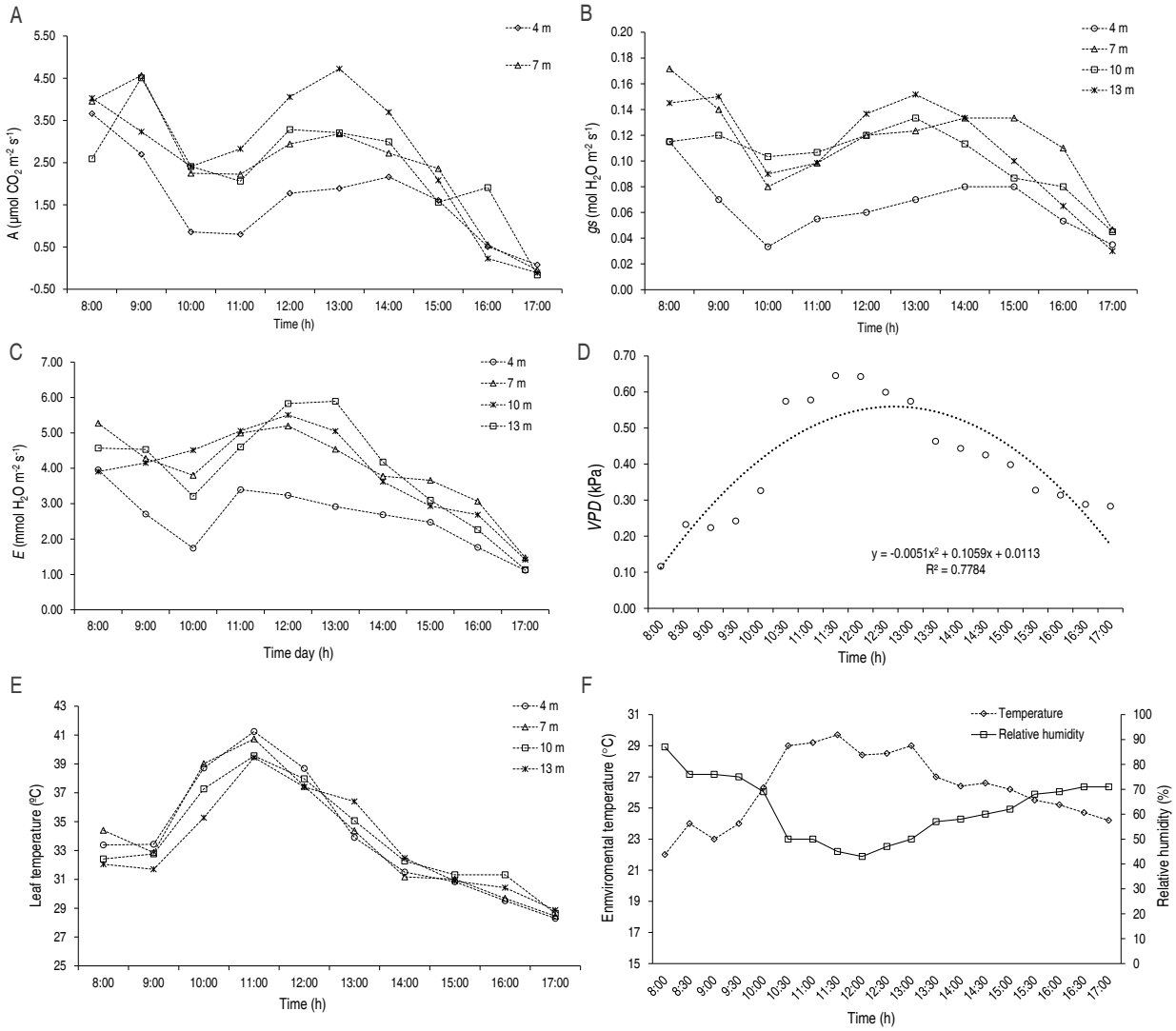


Figure 2. A. Diurnal variation of net photosynthetic rate (*A*); B. Stomatal conductance (g_s); C. Transpiration rates (*E*); D. Vapor pressure deficit *(VPD*) at the top of cocoa; E. Leaf temperature; F. Environmental temperature and relative humidity in cocoa plants at distances of 4, 7, 10, 13 m from Spanish elm trees.

According to Acheampong *et al.* (2013), high *VPD* is directly related to the reduction of photosynthetic activity.

Between 10:00 and 13:00 hours, the leaf temperature in all the cocoa plants exceeded 35 °C (Figure 2E). A temperature that is considered above the optimal (31-33 °C) according to Balasimha *et al.* (1991), who also indicated that the temperature affected the photosynthesis, which is characteristic of tropical species grown in warm, humid tropics. On the other hand, the foliar temperature of plants at 4 m is higher by 1-4 °C than the leaf temperature at 13 m. In contrast, after 11:00 hours, the plants at 13 m exceeded on average by 1 °C the cocoa plants at 4 m.

The *PAR* increased from 9:00 to 11:00 hours but started decreasing from noon to 17:00 hours, where the *PAR* was between 32 to 44 µmol photons m^{-2} s^{-1} (Figure 3A). In the morning, cocoa plants at 13 m receiving the lowest *PAR*, values ranging from 262 to 319 µmol photons m^{-2} s^{-1}, and the highest shade level (67.37 and 72.71 %). At 11:30, all the cocoa plants were submitted to the lowest shade levels because the sun was above the zenith of the cocoa canopy, and the shade trees had little effect on the interception of the radiation. From 13:00 hours, depending on the cocoa plant position, these received different levels of shade, where the position 4 m was the first to receive more shade, and successively the plants to 7, 10, and 13 m (Figure 3B).

According to Balasimha *et al.* (1991), the optimal temperature for tropical species grown in warm, humid tropics is between 31 °C and 33 °C. For cocoa, the optimum temperature range is 20-30 °C. These thermal conditions allow the cocoa crop not only an adequate vegetative development but also a better production, derived from a higher flow of stomatal conductance and greater assimilation of CO_2. Although high environmental temperatures (29.7 °C) were recorded at noon, these did not affect the photosynthetic activity. *A* and g_s presented a direct relationship, i.e., higher stomatal conductance and higher values of carbon fixation were achieved. The values obtained from the gas exchange were higher in this study than those reported by Almeida *et al.* (2014) in *T. cacao* plants but lower than the gas exchange parameter raised for other species of the *Theobroma* genus (*A*, 3.5-8.8 µmol CO_2 m^{-2} s^{-1}; g_s, 0.23-0.108 mol H_2O m^{-2} s^{-1}, and *E*, 0.39-1.63 mmol H_2O m^{-2} s^{-1}).

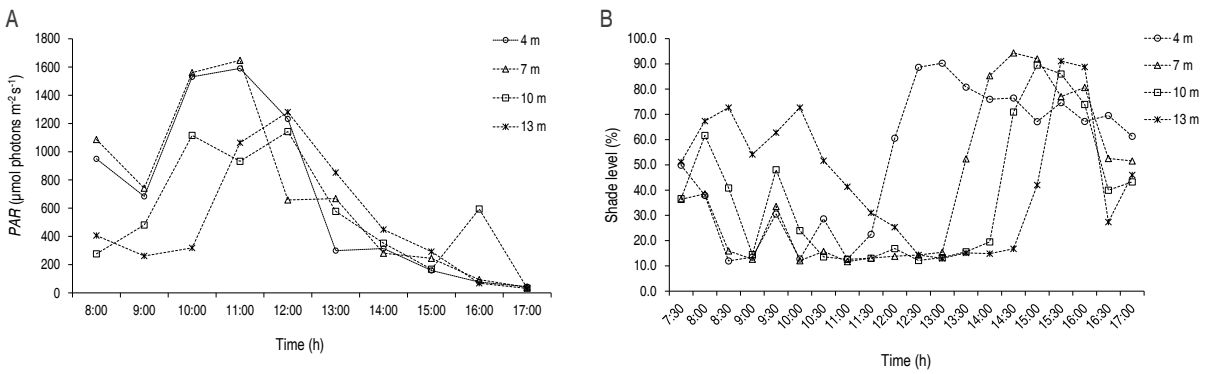


Figure 3. A. Diurnal variation of photosynthetically active radiation (*PAR*) and B. Shade level at the top of *Theobroma cacao* plants at distances of 4 m, 7 m, 10 m, 13 m from Spanish elm trees

Daily integral curve

There were positional variations in the daily integral of *A* ($P<0.05$), g_s ($P<0.05$), and *E* ($P<0.05$). The highest values of these parameters were found in cocoa plants at 13 m from the left edge of the Spanish elm trees, while the lowest results at the opposite side (4 m). Plants at 13 m presented 90,807 µmol CO_2 m^{-2} d^{-1}, corresponding to 77% more CO_2 fixed than plants at 4 m. Similar behavior of the g_s and *E* parameters was observed at 4 m and 13 m plant distance, with more stomatal conductance and transpiration rate in the latter, reaching values of 3,642 mol H_2O m^{-2} d^{-1} and 131,262 mmol H_2O m^{-2} d^{-1}, respectively. Plants from 7 and 10 m always presented intermediated values in all the gas exchange parameters.

The *WUE* was not affected by various shade regimes, whereas the radiation use efficiency was affected. Results indicated that 13 m plant had signed ($P<0.05$) higher *RUE* compared to 4 m plants distance, 250% higher variation in *RUE* (Table 1). Thus, the photosynthetic apparatus of crop species reflects the selection pressure for maximal light absorption under different irradiance, while minimizing the respiratory cost associated with high photosynthetic capacity (Chazdon *et al.*, 1996). In this sense, Suárez Salazar *et al.* (2018) showed that cocoa plants can acclimatize to different levels of radiation, by reducing chlorophyll content and chlorophyll/carotenoid ratio, as well as an increase in chlorophyll a/b ratio, and higher nonphotochemical quenching values, which favors

the dissipation of excess energy in the form of heat and a higher electron transport rate. Depending on the response, photosynthetic performance in the cocoa canopy can be a positive, neutral, or negative way.

Table 1. Daily integral of the net photosynthetic rate (*A*), stomatal conductance (g_s), transpiration rate (*E*), photosynthetically active radiation (*PAR*), water use efficiency (*WUE*), and radiation use efficiency (*RUE*) measured in leaves of *Theobroma cacao* plants at distances of 4 m, 7 m, 10 m, 13 m from Spanish elm trees.

Parameter	*P*	4 m	7 m	10 m	13 m
A (µmol CO_2 m^{-2} d^{-1})	0.0140 **	51,030 b	81,963 ab	83,385 ab	90,807 a
g_s (mol H_2O m^{-2} d^{-1})	0.0020 **	2,076 b	3,711 a	3,396 a	3,642 a
E (mmol H_2O m^{-2} d^{-1})	0.0002 **	84,471 b	132,004 a	130,266 a	131,262 a
PAR (µmol photons m^{-2} d^{-1})	0.01719 ns	2.29×10^7 a	2.32×10^7 a	1.98×10^7 a	1.72×10^7 a
WUE (*A*/*E*)	0.6303 ns	0.60 a	0.62 a	0.64 a	0.69 a
RUE (*A*/*PAR*)	0.0012 **	0.0022 c	0.0035 bc	0.0042 ab	0.0052 a

For each variable, means followed by the same letter are not significantly different using the Tukey's procedure ($P<0.05$).

The highest levels of shade occurred during the afternoon hours. An essential factor to bear in mind when providing shade to the cocoa crop is that it is possible to reach an optimum photochemical activity depending on the shade level (%) to which the cocoa trees are exposed. According to Jaimez *et al*. (2018), cocoa plants can be cultivated with 50% shade since a higher shade and a lower leaf area index affects the ecophysiological response and the yield of cocoa in different environmental conditions. In this study, the plants located at 13 m, presented higher values of gas exchange, confirming what was reported by Gonçalves *et al.* (2005), who indicated that cocoa plants under shady conditions achieved between 33% and 50% higher photosynthetic activity than those plants that had more incidence of radiation. However, Chazdon *et al*. (1996) showed an inverse behavior, where it mentions that plants exposed to higher radiation increase their carbon gain, and in turn, their photoprotection capacity.

CONCLUSIONS

The cocoa plants in the 13 m position presented more favorable environmental conditions with a positive effect over the net photosynthetic rate (*A*), stomatal conductance (g_s), and transpiration rate (*E*) during the day and greater radiation use efficiency (*RUE*). Unlike, the cocoa plants located 4 m away from the Spanish elm trees, achieved the lowest values of the photosynthetic activity. The spacing between shade trees should guarantee favorable conditions. This can be achieved by extending the distance between rows of sowing, of forest trees or by implementing simple rows of the forest species.

ACKNOWLEDGMENT

This publication is derived from the results and information obtained in the development of the project "*Recomendaciones técnicas de sistemas alternativos de producción intensiva y tradicional de cacao con énfasis en arreglos agroforestales*", executed by the Corporación Colombiana de Investigación Agropecuaria - AGROSAVIA, within the framework of the Agenda Dinámica Corporativa, financed with public resources through the Ministerio de Agricultura y Desarrollo Rural (MADR). We thank to Jairo Rojas Molina and Danilo Augusto Monsalve García as national and regional leaders, respectively, of the mentioned project, and to Juan Pablo Gil Restrepo and Juan José Guerra Hincapié for the technical support in the establishment, management and evaluation of the experimental plot. Thanks to the Laboratorio de Ecofisiología de Plantaciones en el Trópico, Departamento de Ciencias Agronómicas, Universidad Nacional de Colombia for its support by Eco physiological instruments.

REFERENCES

Acheampong K, Hadley P, and Daymond AJ. 2013. Photosynthetic activity and early growth of four cocoa genotypes as influenced by different shade regimes under West African dry and

wet season conditions. Experimental Agriculture 49(1): 31-42. doi: 10.1017/S0014479712001007

Almeida A-AF and Valle RR. 2007. Ecophysiology of the cocoa tree. Brazilian Journal of Plant Physiology 19(4): 425-448. doi: 10.1590/S1677-04202007000400011

Almeida A-AF, Gomes FP, Araujo RP, Santos RC, and Valle RR. 2014. Leaf gas exchange in species of the *Theobroma* genus. Photosynthetica 52(1): 16-21. doi: 10.1007/s11099-013-0048-8

Araque O, Jaimez RE, Tezara W, Coronel I, Urich R and Espinosa W. 2012. Comparative photosynthesis water relations growth and survival rates in juvenile Criollo cocoa cultivars (*Theobroma cacao*) during dry and wet seasons. Experimental Agriculture 48(4): 513–522. doi: 10.1017/S0014479712000427

Balasimha D, Daniel EV and Bhat PG. 1991. Influence of environmental factors on photosynthesis in cocoa trees. Agricultural and Forest Meteorology 55(1-2): 15-21. doi: 10.1016/0168-1923(91)90019-M

Baligar VC, Bunce JA, Machado RCR and Elson MK. 2008. Photosynthetic photon flux density carbon dioxide concentration and vapor pressure deficit effects on photosynthesis in cocoa seedlings. Photosynthetica 46(2): 216–221.

Carr MKV and Lockwood G. 2011. The water relations and irrigation requirements of cocoa (*Theobroma cacao* L.): A review. Experimental Agriculture 47(4): 653–676. doi: 10.1017/S0014479711000421.

Chazdon RL, Pearcy RW, Lee DW and Fetcher N. 1996. Photosynthetic responses of tropical forest plants to contrasting light environments. pp. 5-55. In: Mulkey SS, Chazdon RL, Smith AP (eds.). Tropical Forest Plant Ecophysiology. Springer, Boston, MA. 675 p. doi: 10.1007/978-1-4613-1163-8_1

Córdoba-Gaona OJ, Monsalve-García DA, Hernández-Arredondo JD, Guerra-Hincapié JJ, Gil-Restrepo JP, Martínez-Bustamante E and Unigarro-Muñoz CA. 2018. Gas exchange in young *Hevea brasiliensis* (Willd. Ex A. Juss.) Müll. Arg. (Euphorbiaceae) plants in Antioquia (Colombia). Ciencia y Tecnología Agropecuaria 19(1): 91-102. doi: 10.21930/rcta.vol19_num1_art:847

Daymond AJ, Tricker PJ and Hadley P. 2011. Genotypic variation in photosynthesis in cocoa is correlated with stomatal conductance and leaf nitrogen. Biologia Plantarum 55(1): 99–104. doi: 10.1007/s10535-011-0013

De Mendiburu F. 2013. Agricolae: Statistical procedures for agricultural research. R package version 1.1-4. In: Accessed: March 2019.

Do Costa LC, De Almeida F and Valle RR. 2001. Gas exchange, nitrate assimilation and dry-matter accumulation of *Theobroma cacao* seedlings submitted to different irradiances and nitrogen levels. The Journal of Horticultural Science and Biotechnology 76(2): 224-230. doi: 10.1080/14620316.2001.11511355.

Dos Santos MR, Rodrigues Donato SL, Arantes AM, Coelho EF and de Oliveira PM.D 2017. Gas exchange in 'BRS Princesa' banana (*Musa* spp.) under partial rootzone drying irrigation in the north of Minas Gerais, Brazil. Acta Agronómica 66(3): 378-384. doi: 10.15446/acag.v66n3.55056

FAOSTAT. 2017. FAOSTAT Online Database. In: Food and Agriculture Organization of the United Nations, http://www.fao.org/faostat/es/#home Accessed: July 2019.

Galyuon IKA, Mcdavid CR, Lopez FB and Spence JA. 1996. The effect of irradiance level on cocoa (*Theobroma cacao* L.): II. Gas exchange and chlorophyll fluorescence. Tropical Agriculture 73(1): 29–33.

García LJ, Romero CM and Ortiz LA. 2005. Evaluación edafoclimática de las tierras del trópico bajo colombiano para el cultivo de cocoa. Corpoica, Mosquera, 58 p.

Gonçalves JFDC, Barreto DCDS, Santos Junior UMD, Fernandes AV, Sampaio PDTB, and Buckeridge MS. 2005. Growth, photosynthesis and stress indicators in young rosewood plants (*Aniba rosaeodora* Ducke) under different light intensities. Brazilian Journal of Plant Physiology 17(3): 325-334. doi: 10.1590/S1677-04202005000300007

Huang Q and Xiao L. 2010. Calculate the area above and/or below a given reference line using SAS® Data Steps. 9 p. In: SAS Conference Proceedings: Western Users of SAS Software 2010 ,San Diego, California.

ICCO - International Cocoa Organization. 2018. Quarterly Bulletin of Cocoa Statistics, Vol. XLIV, No. 3, Cocoa year 2017/18. In: International Cocoa Organization, https://www.icco.org/about-us/international-cocoa-agreements/cat_view/30-related-documents/46-statistics-production.html Accessed: July 2019.

Jaimez R, Tezara W, Coronel I and Urich R. 2008. Ecofisiología del cocoa (*Theobroma cacao*): su manejo en el sistema agroforestal. Sugerencias para su mejoramiento en Venezuela. Revista Forestal Venezolana 52(2): 253–258.

Jaimez RE, Amores Puyutaxi F, Vasco A, Loor RG, Tarqui O, Quijano G, Jimenez JC and Tezara W. 2018. Photosynthetic response to low and high light of cocoa growing without shade in an area of low evaporative demand. Acta Biológica Colombiana 23(1): 95-103. doi: 10.15446/abc.v23n1.64962

Klich MG. 2000. Leaf variations in Elaeagnus angustifolia related to environmental heterogeneity. Environmental and Experimental Botany 44(3): 171–183. doi: 10.1016/S0098-8472(00)00056-3

Köhler M, Hanf A, Barus H, Hendrayanto and Hölscher D. 2014. Cacao trees under different shade tree shelter: effects on water use. Agroforestry systems 1:63-73. doi: 10.1007/s10457-013-9656-3

Niether W, Armengot L, Andres C, Schneider M, and Gerold G. 2018. Shade trees and tree pruning alter throughfall and microclimate in cocoa (*Theobroma cacao* L.) production systems. Annals of Forest Science 75(38): 16. doi: 10.1007/s13595-018-0723-9

R Core Team. 2017. A Language and Environment for Statistical Computing. In: R Foundation for Statistical Computing. Vienna, Austria. http://www.R-project.org

Ribeiro RV, Machado EC, Santos MG and Oliveira RF. 2009. Photosynthesis and water relations of well-watered orange plants as affected by winter and summer conditions. Photosynthetica 47(2): 215-222. doi: 10.1007/s11099-009-0035-2

Rosenberg NJ, Blad, BL and Verma SB. 1983. Microclimate: The biological environment. Second edition. John Wiley and Sons, Inc., New York. 495 p.

Shiang K-D. 2004. The SAS® Calculations of areas under the curve (UC) for multiple metabolic readings 14 p. In: SAS Conference Proceedings: Western Users of SAS Software 2004. Pasadena, California.

Suárez Salazar JC, Melgarejo LM, Casanoves F, Di Renzo JA, DaMatta FM and Armas C. 2018. The photosynthetic response of cocoa photosynthesis limitations in cocoa leaves under different

agroforestry systems in the Colombian Amazon. PLoS ONE 13(11): e0206149. doi: 10.1371/journal.pone.0206149.

Tezara W, Urich R, Jaimez R, Coronel I, Araque O, Azocar C and Chacón I. 2016. Does Criollo cocoa have the same ecophysiological characteristics than Forastero? Botanical Sciences 94(3): 563-574. doi: 10.17129/botsci.552

Wessel M. 1985. Shade and nutrition. pp. 18-23. In: Lass RA and Wood GAR (eds.). Cocoa production: present constraints and priorities for research. World Bank technical paper (WTP39), Washington. 120p. doi: 10.1002/9780470698983.ch7

Research article

http://www.revistas.unal.edu.co/index.php/refame

Revista Facultad Nacional de Agronomía

Phenological patterns of defoliation and refoliation processes of rubber tree clones in the Colombian northwest

Patrones fenológicos de los procesos de defoliación y refoliación en clones de caucho en el noroeste de Colombia

UNIVERSIDAD NACIONAL DE COLOMBIA

doi: 10.15446/rfnam.v73n3.80546

Juan José Guerra-Hincapié[1]*, Oscar de Jesús Córdoba-Gaona[2], Juan Pablo Gil-Restrepo[1], Danilo Augusto Monsalve-García[1], Juan David Hernández-Arredondo[1] and Enrique Guillermo Martínez-Bustamante[2]

ABSTRACT

The knowledge of the defoliation-refoliation process in rubber cultivation allows the development of management strategies in the production system to improve rubber yield. The objective of this study was to determine the intensity and duration of defoliation-refoliation of rubber clones FX 3864, IAN 710 and IAN 873 in the municipality of Tarazá and the FX 3864 and IAN 873 clones in the municipality of Nechí (northwestern Colombia). From October 2015 to June 2016, the measurements of the necromass were carried out in each location for each clone. The light environment was quantified, employing the hemispheric photographs technique to estimate canopy openness percentage (CO) and leaf area index. The assessed weeks were grouped by Principal Component Analysis (PCA) based on the original phenology and climatic variables. The defoliation-refoliation process was analyzed descriptively using graphical representations of the trend for the phenological variables that best described this process. The relationship between climatic and phenological variables in the period evaluated was evidenced; the rainfall was the most critical climatic characteristic in the induction of the defoliation process. The leaf area index was reduced to a minimum value in February, with values of 0.52 for IAN 710 clone in Tarazá, and 0.64 for the IAN 873 clone in Nechí, which corresponded to the highest defoliation stage in both locations. The refoliation period was short (4 to 6 weeks) and occurred during the dry season for all the clones in both places.

Keywords:
Hevea brasiliensis
Leaf area index
Phenology
Tropical species

RESUMEN

El conocimiento del proceso de defoliación–refoliación en el cultivo del caucho permite desarrollar estrategias de manejo en el sistema productivo encaminadas a mejorar el rendimiento de caucho. El objetivo de este estudio fue determinar la intensidad y duración de la defoliación-refoliación de los clones de caucho FX 3864, IAN 710 e IAN 873 en el municipio de Tarazá y los clones FX 3864 e IAN 873 en Nechí, ubicados en el noroccidente de Colombia. Durante el período de defoliación-refoliación entre octubre de 2015 y junio de 2016, la recolección y cuantificación de necromasa se llevó a cabo en cada plantación y para cada clon. El ambiente lumínico se cuantificó empleando la técnica de fotografías hemisféricas para estimar el porcentaje de apertura del dosel (CO) y el índice de área foliar en el cultivo de caucho. Para agrupar las semanas de medición por los procesos fenológicos ocurridos, se realizó un Análisis de Componentes Principales (PCA) basado en las variables de fenología y de clima. La evolución del proceso de defoliación-refoliación se analizó descriptivamente mediante representaciones gráficas de tendencia para las variables fenológicas que mejor describieron este proceso. Se evidenció la relación entre las variables climáticas y fenológicas en el período evaluado, siendo la lluvia, la característica climática más crítica en la inducción del proceso de defoliación. El índice de área foliar se redujo a un valor mínimo en febrero, con valores de 0.52 para el clon IAN 710 en Tarazá, y 0.64 para el clon IAN 873 en Nechí, que correspondió a la etapa de defoliación más alta en ambos lugares. La duración del período de refoliación fue corta (4 a 6 semanas) y se produjo durante la estación seca para todos los clones en ambos lugares.

Palabras clave:
Hevea brasiliensis
Índice de área foliar
Fenología
Especies tropicales

[1] Corporación Colombiana de Investigación Agropecuaria – AGROSAVIA. Centro de Investigación El Nus. Corregimiento de San José del Nus, San Roque, Antioquia, Colombia.

[2] Facultad de Ciencias Agrarias. Univesidad Nacional de Colombia. AA. 1779, Medellín, Colombia.

* Corresponding author: <jjguerra@agrosavia.co>

Received: October 31, 2019; Accepted: July 9, 2020

Rev. Fac. Nac. Agron. Medellín 73(3): 9293-9303. 2020

ISSN 0304-2847 / e-ISSN 2248-7026

Rubber tree [*Hevea brasiliensis* (Willd. Ex A. Juss.) Müll. Arg.] is a perennial plant species belonging to the Euphorbiaceae family and is native to the Amazon River basin. This zone is characterized by having an Intertropical environment with high rainfall, temperature, and radiation (Priyadarshan, 2017). The rubber tree is cultivated mainly in the equatorial zone between 10° S and 10° N (Meenakumari *et al*., 2018). In northwestern Colombia, one of the most important producing areas is called *El Cordón Cauchero-Cacaotero*. This zone has the largest number of producers (79%) in the department of Antioquia (Colombia), including the localities Tarazá and Nechí, which are representative of this area (CCC, 2015).

Biological events in plants are sensitive to climatic changes (Richardson *et al*., 2013), and their phenological behavior is influenced by factors such as temperature, rainfall, radiation, and soil nutrients (Zhai *et al*., 2017; Fu *et al*., 2015). In the specific case of rubber, its growth is annual, and the defoliation period can vary depending on the genetic material, planting density (Righi *et al*., 2001), and altered environmental conditions (Priyadarshan, 2017). According to Guyot *et al*. (2008), the period of refoliation lasts approximately one month. It includes the emergence of the leaf to the mature leaf stage described by Lieberei (2007) as stage D.

Paranjothy (2018) mentioned that rubber trees respond to drought stimuli, which generate variation in leaf phenology at different times and locations. However, Priyadarshan (2017) indicated that the phenological phases respond to the latitudinal position where plantations are located. In the north of the equator, the defoliation occurs in February-March, mainly associated with low humidity and high transpiration rate, while in the south of the equator in September-October, after winter with low temperature and water deficiency.

According to Carr (2012), defoliation occurs in trees older than three years. It is induced by dry or less humid climates where trees can remain almost leafless for up to four weeks. In addition, radiation in tropical areas can cause defoliation and refoliation (Borchert *et al*., 2015).

In tropical climates, rubber plants produce latex throughout the year with a marked reduction in the defoliation and refoliation periods since photosynthesis is restricted (Righi *et al*., 2001), which decreases the amount of reserves (carbohydrates) and total production (Simbo *et al*., 2013). The periods of defoliation, refoliation and subsequent rubber tree flowering occur at the same time of the year for all clones. Still, the precocity, homogeneity and speed of the event diverge substantially (Guyot, 2008).

It is necessary to know the behavior of natural rubber plantations and the functioning of different genotypes in rubber-producing zones of Colombia (Córdoba-Ganoa *et al*., 2018) to determine relationships between climatic characteristics with defoliation-refoliation patterns and their dynamics with the occurrence of diseases. According to Guyot and Le Guen (2018), refoliation is the phenological phase that is most susceptible to the South American Leaf Blight (SALB) caused by the fungus *Pseudocercospora ulei* (P. Henn.) V. Arx (Hora Junior *et al*., 2014), which is a major limiting factor for latex production by significantly reducing the tree leaf area (Jaimes and Rojas, 2011). It is hypothesized that the defoliation – refoliation process occurs at the same time for all the studied clones and localities but showing different durations and intensities, with the rainfall regime as the principal trigger for phenological events. That is why this study aimed to evaluate the intensity and duration of the defoliation-refoliation processes of rubber tree clones FX 3864, IAN 873 and IAN 710 in the productive stage in two northwestern Colombian municipalities (Tarazá and Nechí).

MATERIALS AND METHODS

This research was conducted as an observational study in two rubber tree commercial plantations under production, in the locality of Tarazá, administrative section of Santa Clara, and the locality of Nechí, administrative section of Quebrada La Cienaga, in Antioquia (Colombia).

In Tarazá (7°30' N, 75°30' W, 130 masl) monoclonal plots of 0.3 ha with the rubber clones FX 3864 (FX: Ford Cross), IAN 873 and IAN 710 (IAN: Instituto de Pesquisas Agropecuarias do Norte) were monitored. These were planted at 2.8 m between plants and 7 m between rows in the year 1998 and are under tapping since 2011. In Nechí (7°53' N, 74°50' W, 70 masl) monoclonal plots of 0.3 ha with the rubber clones FX 3864 and IAN 873 were monitored. These were planted at 2.8 m between plants and 7 m between rows in the year 2006, and are under tapping since 2015.

In both localities, the rainfall regime is monomodal with an annual mean rainfall of 4079 mm in Tarazá and 4058 mm in Nechí, the dry period occurs from December to March and the rainy season occurs from April to November. The rubber plantations are in Tarazá's flatlands and in Nechí's hilly landscape. In both locations, the soils are classified as Ultisols, with high acidity and low natural fertility (Villa *et al.*, 2017). During this study, the rubber trees were tapped downward on half spiral cuts, at three daily frequencies, six days in tapping followed by one day of rest, without stimulation.

During the defoliation-refoliation period between October 2015 and June 2016, the collection and quantification of the necromass (leaves, flowers, fruit peels, branches and seeds) were carried out in each plantation and for each clone. The collection was performed in five sampling units randomly distributed, each consisting of three necromass collector nets of 1 m^2, 0.7 m from the soil surface, collecting necromass from four rubber trees, as shown in Figure 1. The collected necromass was dried in a Thermolab TO 90 S/G oven at 50 °C until a constant weight was reached. The dry weight (g) of leaves (DWL), flowers (DWF), fruit epicarp (DWP), branches (DWB) and seeds (DWS) were established independently. The quantification of the foliar area (FA) trapped in the collector nets was carried out employing the leaf area portable meter Licor® 3000C.

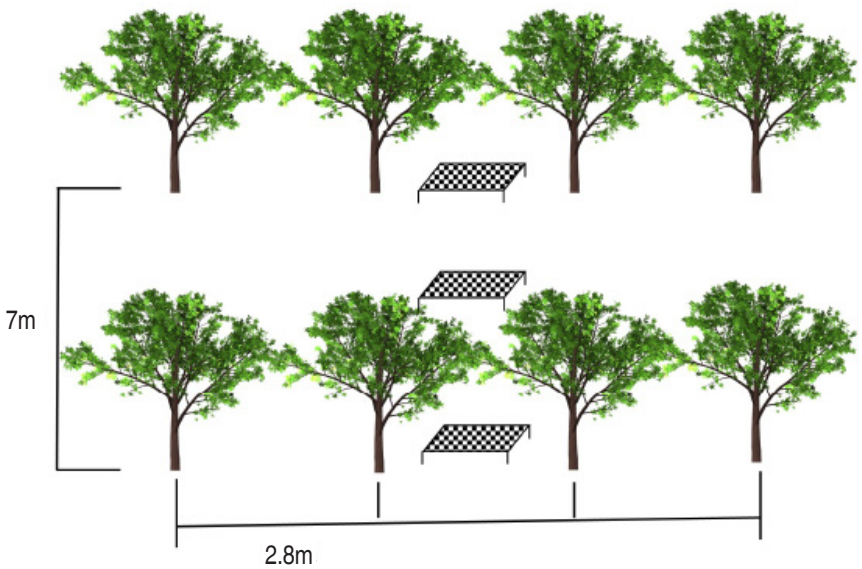


Figure 1. Graphic representation of the location of the necromass collector nets in each sampling unit.

The light environment was quantified, employing the hemispherical photography technique to the canopy of the crop (Chazdon and Field, 1987). A GoPro Hero + ® camera (GoPro Inc.) was used on each of the necromass capturing sites (meshes). The photographic records were directed to the canopy. The photographs taken were analyzed with the Gap Light Analyzer software (GLA) (Frazer *et al.*, 1999) to estimate the canopy openness percentage (CO) and leaf area index. These measurements were made by inserting a circle into each photography and dividing it into six concentric rings and evaluating the variables on ring 4 (LAI4) and 5 (LAI5). The evaluations were conducted monthly between October 2015 (week 44 of 2015) and June 2016 (week 24 of 2016), except between October and February, where the frequency was biweekly due to the accelerated increase of necromass.

The following climatic variables were recorded in each location: average relative humidity (HR) (%), minimum relative humidity (%), maximum relative humidity (%), average temperature (TEM) (°C), minimum temperature (°C), maximum temperature (°C), photosynthetically active

radiation (PAR) (µmol photon m^{-2} s^{-1}) and accumulated rainfall (RAINF) (mm $week^{-1}$), through the weekly recording in Spectrum® portable weather stations of the Watchdog 2900 ET series., the evapotranspiration was calculated using the Hargreaves equation (Allen *et al.*, 2006) to perform the climatic characterization of each locality, where evapotranspiration (ETo) was equivalent to ETc because according to Carr (2012) the coefficient of rubber crop is 1.

In order to group the assessed weeks by the occurring phenological processes, a Principal Component Analysis (PCA) was carried out based on the original phenology and climatic variables described above. From this, a biplot representation with 95% confidence ellipses was elaborated, using the statistical procedure "ggbiplot" of the statistical software R Project (R core Team, 2017).

The evolution of the defoliation-refoliation process was analyzed descriptively using graphical representations of the trend for the phenological variables that best described this process, for each clone and locality. The observed co-occurrence of phenological and climatic events was discussed.

On the other hand, to compare the defoliation and refoliation processed by clone (considered as fixed effects) in each location, the following response variables were used: dry weight of leaves, flowers, seeds, and leaf area index. For their analysis, the non-parametric Kruskal-Wallis rank test (95% confidence level) and the post hoc tests for multiple comparisons were implemented by the Conover method (Conover, 1999; Pohlert, 2014), using the statistical package "agricolae" of the statistical R Project software (De Mendiburu, 2013).

RESULTS AND DISCUSSION

From the PCA (Figure 2), all data contained in 14 variables were summarized in two principal components (PC1 and PC2). However, this reduces the amount of information and it improves interpretability by plotting all variables in two dimensions, allowing a better comprehension of a complex biological phenomenon such as phenology with multiple factors involved. The amount of variance explained for the components PC3 to PC14 does not compensate the interpretability lost because of the increased dimensionality. The PCA showed an important relationship between climatic and phenological variables

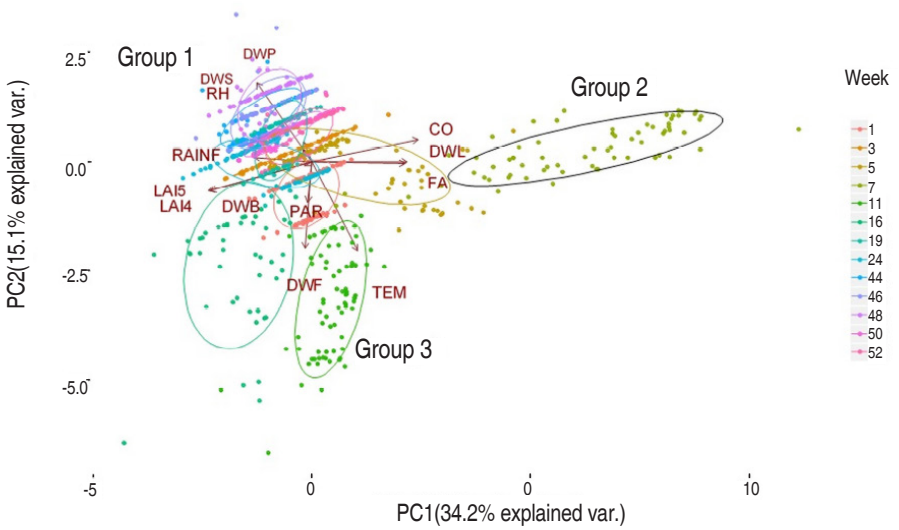


Figure 2. Biplot representation of the relationship between defoliation-refoliation processes variables and the climate variables in weeks 44, 46, 48, 50, 52 of 2015 and weeks 1, 3, 5, 7, 11, 16, 19 and 24 of 2016. LAI4 and LAI5 (leaf area index in the fourth and fifth ring), CO (canopy openness), FA (foliar area), DWL (dry weight of the leaves), DWB (dry weight of branches), DWF (dry weight of the flowers), DWP (dry weight of the fruit peel), DWS (dry weight of the seed), TEM (temperature), PAR (photosynthetically active radiation), RAINF (rainfall) and RH (relative humidity).

according to the evaluation period where the processes of defoliation, refoliation, flowering and fruit dehiscence occurred.

The PCA identified three stages: transition, maximum defoliation and refoliation, represented as groups of ellipses without interception between them (Groups 1, 2, and 3; Figure 2), that synthesize the behavior of the defoliation-refoliation period based on the two first principal components (PC1 and PC2), which explained 48.9% of the variation. The PCA findings were similar to those reported by Liyanage *et al*. (2018), who observed in five rubber clones studied in Southwest China (22° N) that defoliation occurred between December and January, refoliation between January and February and flowering in March to April, while the trees were in foliar stage D according to the scale proposed by Lieberei (2007) from April to December.

The first stage (1) comprising weeks 44, 46, 48, 50, and 52 of 2015 and 1, 3, 5, 16, 19, and 24 of 2016 corresponds to rainy seasons of high relative humidity and with a higher leaf area index. In this group, weeks 44-48 stood out due to seed fall, week 5 as a transition time towards the defoliation phase, and, in week 16, trees showed a higher leaf area index; moreover, these were in the foliar phenological stage D. Whereas Liyanage *et al*. (2018) found that precipitation is less critical than sunshine exposure time in determining the timing of rubber phenological phases, the rubber defoliation process in Tarazá and Nechí was influenced by the decrease in rainfall intensity. In the second stage (week 7), the highest canopy openness, dry weight and leaf area of the fallen leaves, as well as the lowest leaf area indexes, were obtained, which was associated with the highest defoliation period. Carr (2012) reported that this defoliation stage is induced by dry or less humid climates, where trees can remain almost leafless for up to four weeks. The third stage (week 11) was the refoliation period after the leaf loss and the flowering stage. This period is associated with a higher dry weight of flowers, high radiation, and high temperature; the main flowering season of rubber occurs between March and April in the northern hemisphere, preceded by the end of the defoliation – refoliation process, which happens in January to February, as was shown by Priyadarshan (2017).

In this work, rainfall was the most important climatic characteristic in the induction of the defoliation stage. The most significant foliage loss was generated from week 7. Similarly, Priyadarshan (2017) commented that the defoliation stage is a phenomenon in which the plant, in a stress condition due to water deficit, induces leaf fall as a strategy to reduce transpiration rates and ensures its reproduction. Furthermore, Li *et al.* (2016) reported that the foliar abscission and senescence are attributed to drought stress. Both the photosynthetically active radiation and temperature accumulated did not have a defined pattern. Therefore, the relationship of these two variables with a specific phenological process was not evident, although Borchert *et al.* (2015) and Priyadarshan (2017) mentioned that radiation and temperature have an important relationship in the phenology of the plant.

Figure 3 shows the evolution of the leaf area index of the canopy over time. The leaf area index was reduced, for all clones and localities, to a minimum value in week 7 (February) of 2016, with values of 0.52 for IAN 710 clone in Tarazá and 0.64 for the IAN 873 clone in Nechí. It corresponded to the highest defoliation stage in both locations. In Nechí for IAN 873, the reduction in the leaf area index occurred from week 5, reaching a minimum (0.63) in week 7 (Figure 4). Regarding FX 3864 clone, defoliation occurred specifically in week 7, which indicates that this clone is more efficient in terms of conservation of its leaf area than IAN 873; meanwhile, the recovery of its foliage was similar and occurred at week 11 for both clones. For week 7 in both clones, canopy openness of 60% and 50%, respectively, were obtained. The variables that best described the phenological behavior of the defoliation-refoliation processes of the rubber plants were the leaf area index of the fourth ring (LAI4), dry weight of the leaves, and percentage of canopy openness. These variables showed weeks 5 and 7 as the period of maximum defoliation, which was similar to what has been reported by Lin *et al.* (2018), who mentioned that the defoliation in rubber tree plantations of southeastern China was characterized by having a marked reduction in the leaf area index (0.5) during January.

The highest dry weight of the leaves for the FX 3864 clone was observed two weeks before IAN 873, without reflecting the decrease of the leaf area index. The defoliation stage in Tarazá was limited to week 7 for all

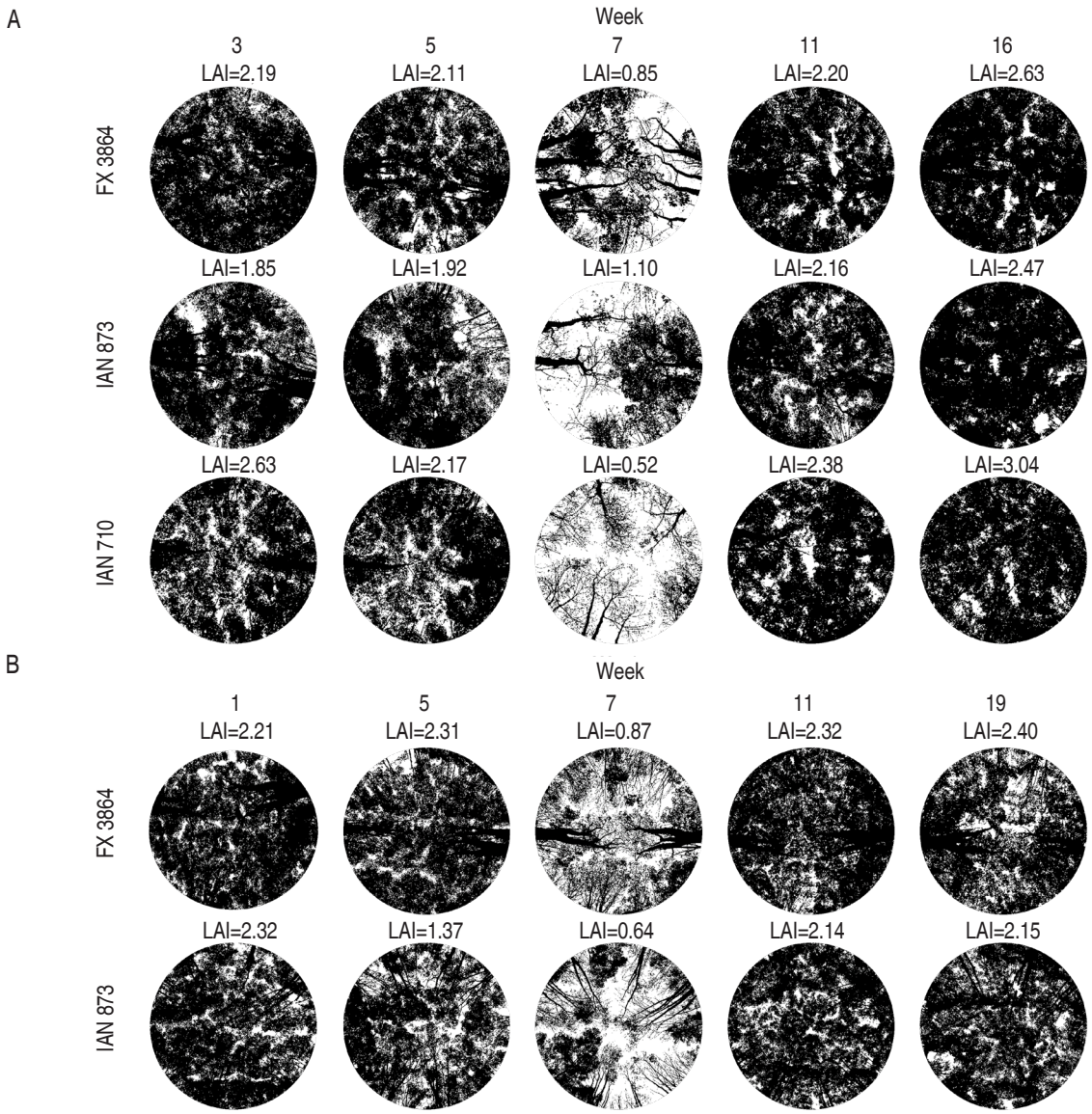


Figure 3. Evolution of the leaf area index (LAI) in the defoliation-sprouting season quantified in 2016 during the weeks (W) 3, 5, 7, 11 and 16 for clones FX 3864, IAN 873, and IAN 710 in A. Tarazá; and the weeks (W) 1, 5, 7, 11, and 19 for clones FX 3864 and IAN 873 in B. Nechí.

genetic materials. The lowest values (0.52) of the leaf area index and the highest openness of the canopy (67%) were found in IAN 710 clone. Concerning the accumulated leaf biomass, it was 3.2 and 1.6 t ha^{-1} for FX 3864 and IAN 873 clones, respectively, in Nechí. In Tarazá, it was 2.3 t ha^{-1} for both FX 4098 and IAN 873, and 2.0 t ha^{-1} for IAN 710. However, the accumulated biomass found during the whole defoliation process in this study was higher than what was reported by Meti *et al.* (2014), who mentioned that in a rubber plant production system in India with the RRII 105 clone, litter values ranged between 1.67-1.9 t ha^{-1} year^{-1}, which constitutes a contribution of minerals to the soil for the nutrient cycling process.

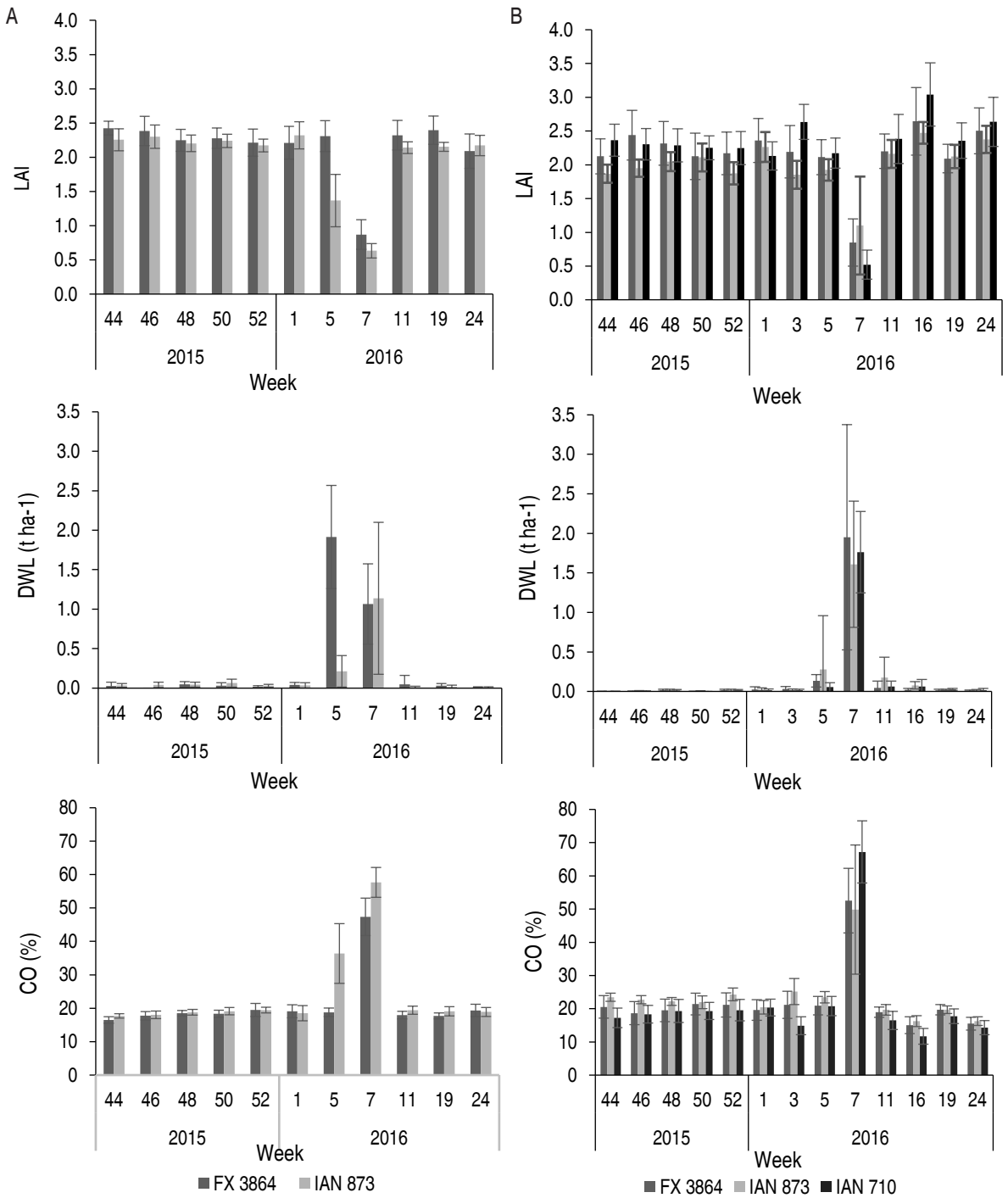


Figure 4. Behavior of leaf area index (LAI), dry weight of leaves (DWL) and canopy openness (CO) of rubber clones FX 3864, IAN 873, and IAN 710 in A. Nechí and B. Tarazá.

Regarding the climatic factors (Figure 5), the minimum temperature was similar in both locations (approximately 24 °C). However, the photosynthetically active radiation averaged per week (1,145 µmol photon m^{-2} s^{-1}) and the maximum temperature (38.5 °C) were higher in Nechí compared to Tarazá (904 µmol photon m^{-2} s^{-1} and 36 °C) (Figures 5A and 5B). In Nechí, the dry period was between December to March (week 50-2015 to week

16-2016) with a negative water balance (Figure 5C). In Tarazá, the occurrence of rains did not have the same pattern; a dry season was identified with a negative water balance in February and March (Figure 5D). In Nechí, IAN 873 and FX 3864 clones were subjected to a reduction in rainfall intensity of 97% from November to January to reach the state of greatest defoliation. As for Tarazá, defoliation was synchronized in all clones, and only a 31% reduction in rainfall intensity in the same months was sufficient for its occurrence. The occurrence of the dry season (negative water balance) before maximum defoliation was evident

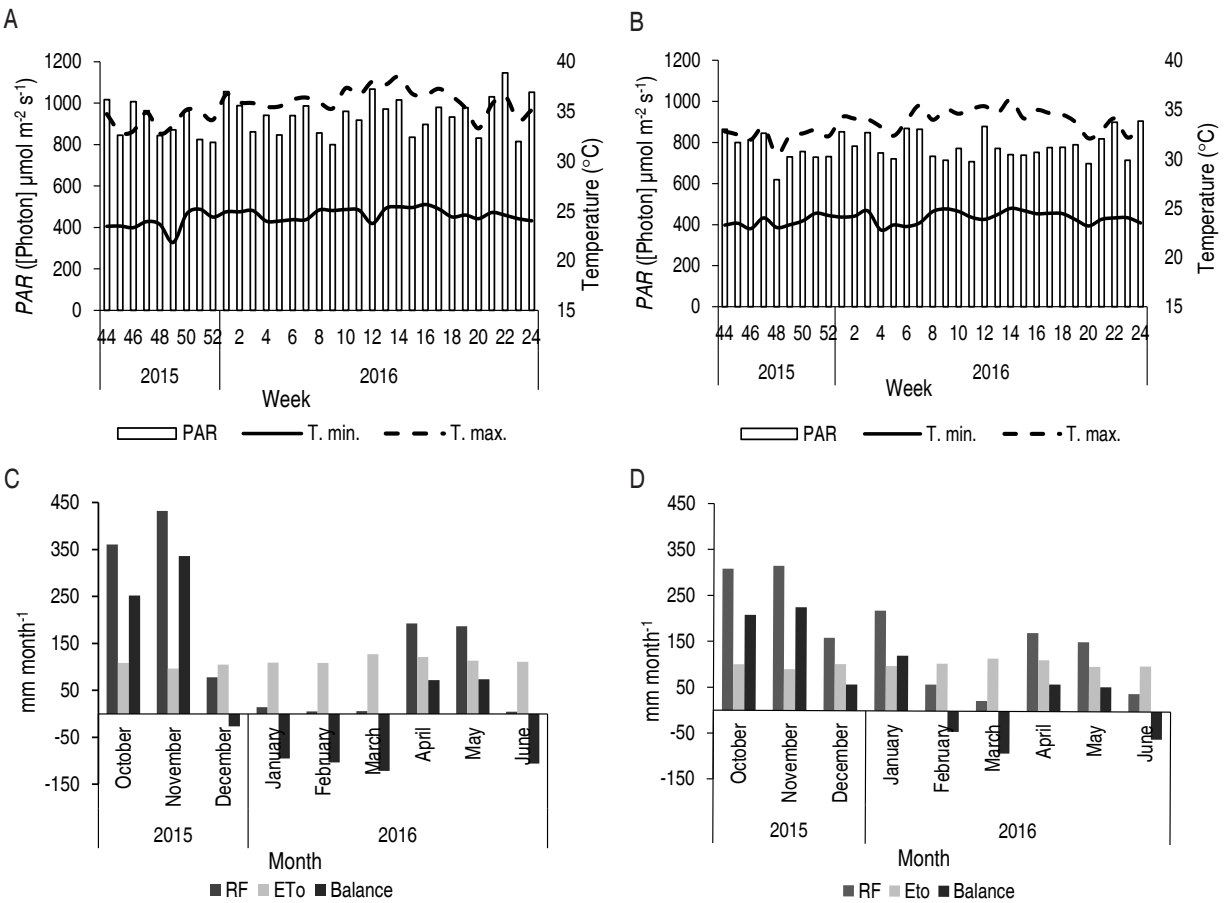


Figure 5. Behavior of photosynthetically active radiation (PAR), minimum and maximum temperatures in A. Nechí and B. Tarazá in weeks 44-52 of 2015 and weeks 1-24 of 2016. Rainfall (RF), evapotraspiration (ETo) and water balance in C. Nechí and D. Tarazá between October 2015 and June 2016.

in Nechí. However, a reduction in the intensity of rainfall was enough to trigger the phenological stage in Tarazá. This climatic factor could generate faster and more uniform defoliation (Gasparotto *et al.*, 2012).

Concerning the comparison of genetic materials (Table 1), in Tarazá, the clone with the highest leaf area index was IAN 873, and the lowest was IAN 710. The smaller leaf area recorded in IAN 710 clone was the result of higher defoliation during the evaluation period. In Nechí, the clone with the highest leaf area index was FX 3864, although the dry weight of the detached leaves was similar among clones. The intensity of the flowering evidenced by the dry weight of flowers was similar in all the clones, albeit the dry weight of seeds was higher for IAN 873 in Nechí. In this sense, Righi *et al.* (2001) mentioned that the production of rubber has a high correlation with the leaf area index since a more extended period of defoliation would be

associated with a decrease in latex production. According to Guyot (2008), homogeneous and short defoliation processes generate an escape condition from phytosanitary problems, especially the South American Leaf Blight (SALB) disease. However, clones with greater susceptibility to SALB with a lower percentage of retained leaves, at the end of the annual defoliation–refoliation, could present the most intense SALB signs and symptoms (Sterling *et al*., 2019).

Table 1. Comparison of mean ranges employing the Kruskal-Wallis test and the Conover post hoc tests for the variables dry weight of leaves (DWL), dry weight of flowers (DWF), leaf area index (LAI) and dry weight of seeds (DWS) of clones FX 3864, IAN 873 and IAN 710 in the localities of Nechí and Tarazá.

Location	Clone	DWL (kg m^{-2})		DWF (kg m^{-2})		LAI		DWS (kg m^{-2})	
		Mean	Range*	Mean	Range*	Mean	Range*	Mean	Range*
Tarazá	FX 3864	0.1740	275.92 b	0.0097	284.64 a	2.16	301.31 b	0.0009	292.01 a
	IAN 873	0.1736	284.82 b	0.0258	293.63 a	2.01	350.41 a	0.0004	296.49 a
	IAN 710	0.1569	318.26 a	0.0417	300.73 a	2.26	227.27 c	0.0013	290.49 a
Nechí	FX 3864	0.2856	156.06 a	0.0199	160.71 a	2.18	180.59 a	0.0005	155.51 b
	IAN 873	0.1035	163.86 a	0.0098	159.30 a	2.03	139.79 b	0.0037	164.41 a

* Different letters mean statistical differences with 95% confidence level for the variable's mean ranges within each location

The duration of the refoliation period was short (4-6 weeks) for all the clones in both locations. It occurred in the dry season, which was similar to Silva *et al.* (2012), who mentioned that despite a water restriction, the plants could develop their foliage. However, Rivano *et al*. (2016) indicated that the defoliation and refoliation process could be extended up to 20 weeks. This short period (4 to 6 weeks) required by rubber trees to complete the defoliation-refoliation cycle is considered an advantage of rubber cultivation in the subregion of Bajo Cauca since the trees quickly recover the foliage and have the capacity to increase the latex production. Similar results were found by Maeght *et al*. (2015), who also mentioned that although the growth of the secondary roots stops during the dry season, a variable quantity of the water demanded is supplied by deeper roots. However, it is important to continue with other studies that allow us to establish whether this duration affects the lifespan and the occurrence of the dry panel and total solids content in rubber trees, and its relationship with the most critical leaf diseases.

CONCLUSIONS

Rubber tree cultivation in northwestern Colombia showed three phenological stages that groups the defoliation and refoliation events, such as maximum defoliation (minimum leaf area index), the transition stage (beginning of abscission and leaf regrowth) and refoliation (maximum index of leaf area and flowering). The defoliation stage was mainly promoted by a reduction in rainfall intensity on the municipalities of Nechí and Tarazá. Furthermore, the refoliation stage in the plantations in Tarazá and Nechí took place in the dry season, was short (4-6 weeks) and synchronized for all the clones.

ACKNOWLEDGMENTS

The results and information of this publication were obtained from the development of the project "Sistemas de apoyo para el seguimiento y gestión del manejo en plantaciones de caucho, a partir de componentes climáticos, edáficos, fitosanitarios y fisiológicos de plantaciones establecidas en núcleos productivos", executed by the Corporación Colombiana de Investigación Agropecuaria - AGROSAVIA, within the framework of the *Agenda Dinámica Corporativa*, financed with public resources through the Ministerio de Agricultura y Desarrollo Rural (MADR). The authors thank Albert Julesmar Gutiérrez Vanegas as national leader of the mentioned project, the Laboratorio de Ecofisiología de Plantaciones en el Trópico, Departamento de Ciencias Agronómicas, Universidad Nacional de Colombia for its Eco physiological instruments, and to the rubber producers' associations ASCULTICAUCHO and ASCABIA for supporting this study.

REFERENCES

Allen RG, Pereira LS, Raes D y Smith M. 2006. Evapotranspiración del cultivo: Guías para la determinación de los requerimientos de agua de los cultivos. Estudio FAO riego y drenaje 56. FAO. Roma, Italia. 322 p.

Borchert R, Calle Z, Strahler AH, Baertschi A, Magill RE, Broadhead JS, Kamau J, Njoroge J and Muthuri C. 2015. Insolation and photoperiodic control of tree development near the equator. New Phytologist 205(1): 7-13. doi: 10.1111/nph.12981

Carr MKV. 2012. The water relations of rubber (*Hevea brasiliensis*): a review. Experimental Agriculture 48(2): 176–193. doi: 10.1017/S0014479711000901

CCC - Confederación Cauchera Colombiana y Corporación Colombiana de Investigación Agropecuaria. 2015. Informe Final. Banco de datos estructurado, ajustado, homogenizado como insumo del sistema de información geográfico (SIG-CAUCHO). Ministerio de Agricultura y Desarrollo Rural. 57 p.

Chazdon RL and Field CB. 1987. Photographic estimation of photosynthetically active radiation: evaluation of a computerized technique. Oecología 73(4): 525-53. doi: 10.1007/BF00379411

Conover WJ. 1999. Practical Nonparametric Statistics. Third edition. Wiley, New York. 592 p.

Córdoba-Ganoa O, Monsalve-García D, Hernández-Arredondo JD, Guerra-Hincapié JJ, Gil-Restrepo JP, Martínez-Bustamante E and Unigarro-Muñoz CA. 2018. Gas exchange in young *Hevea brasiliensis* (Willd. Ex A. Juss.) Müll. Arg. (*Euphorbiaceae*) plants in Antioquia (Colombia). Ciencia y Tecnología Agropecuaria 19(1): 91-102. doi: 10.21930/rcta.vol19_num1_art:540

De Mendiburu F. 2013. Agricolae: Statistical procedures for agricultural research. R package version 1.1-4. In: https://cran.r-project.org/web/packages/agricolae/ Accessed: March 2019.

Frazer GW, Canham CD and Lertzman KP. 1999. Gap Light Analyzer (GLA), Version 2.0: Imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs, user's manual and program documentation. Simon Fraser University, Burnaby, British Columbia, and the Institute of Ecosystem Studies, Millbrook, New York. 36 p.

Fu YH, Piao S, Vitasse Y, Zhao H, De Boeck HJ, Liu Q, Yang H, Weber U, Hänninen H and Janssens IA. 2015. Increased heat requirement for leaf flushing in temperate woody species over 1980–2012: effects of chilling, precipitation and insolation. Global Change Biology 21(7): 2687–2697. doi: 10.1111/gcb.12863

Gasparotto L e Pereira RJC. 2012. Doenças da seringueira no Brasil. Segunda edição EMBRAPA Amazônia Occidental. Brasília, DF. 255 p. https://livimagens.sct.embrapa.br/amostras/00084580.pdf Consulta: diciembre 2018.

Guyot J, Cilas C and Sache I. 2008. Influence of host resistance and phenology on South American Leaf Blight of the rubber tree with special consideration of temporal dynamics. European Journal Plant Pathology 120(2): 111–124. doi: 10.1007/s10658-007-9197-6

Guyot J and Le Guen V. 2018. A review of a century of studies on South American Leaf Blight of the rubber tree. Plant Disease 102(6): 1–14. doi: 10.1094/PDIS-04-17- 0592-FE

Hora Junior BT, De Macedo DM, Barreto RW, Evans HC, Mattos CRR, Maffia LA and Mizubuti ES. 2014. Erasing the past: A new identity for the damoclean pathogen causing South American leaf blight of rubber. PloS One 9(8): e104750. doi: 10.1371/journal.pone.0104750

Jaimes YY y Rojas J. 2011. Enfermedades foliares del caucho (*Hevea brasiliensis* Muell. Arg.) establecido en un campo clonal ubicado en el Magdalena Medio Santandereano (Colombia) Ciencia y Tecnología Agropecuaria 12(1): 65-76. doi: 10.21930/rcta.vol12_num1_art:216.

Liyanage KK, Khan S, Ranjitkar S, Yu H, Xu J, Brooks S, Beckschäfer P and Hydel KD. 2017. Evaluation of key meteorological determinants of wintering and flowering patterns of five rubber clones in Xishuangbanna, Yunnan, China. International Journal of Biometeorology 63(5): 617-625. doi: 10.1007/s00484-018-1598-z

Li Y, Lan G and Xia Y. 2016. Rubber trees demonstrate a clear retranslocation under seasonal drought and cold stresses. Frontiers in Plant Science 7:1907. doi: 10.3389/fpls.2016.01907

Lieberei R. 2007. South American leaf blight of the rubber tree (*Hevea* spp.): new steps in plant domestication using physiological features and molecular markers. Annals of Botany 100(6): 1125-1142. doi: 10.1093/aob/mcm133

Lin Y, Zhang Y, Zhao W, Dong Y, Fei X, Song Q, Sha L, Wang S and Grace J. 2018. Pattern and driving factor of intense defoliation of rubber plantations in SW China. Ecological Indicators 94(1): 104-116. doi: 10.1016/j.ecolind.2018.06.050

Maeght JL, Gonkhamdee S, Clément C, Isarangkool N, Ayutthaya S, Stokes A and Pierret A. 2015. Seasonal Patterns of fine root production and turnover in a mature rubber tree (*Hevea brasiliensis* Müll. Arg.) stand- differentiation with soil depth and implications for soil carbon stocks. Frontiers in Plant Science 6: 1–11. doi: 10.3389/fpls.2015.01022

Meenakumari T, Meenattoor JR, Thirunavoukkarasu M, Vinod KK, Krishan B, Gireesh T and Jacob J. 2018. Dynamics of long-term adaptive responses in growth and rubber yield among *Hevea brasiliensis* genotypes introduced to a dry sub-humid climate of Eastern India. Industrial Crops and Products 119: 294–303. doi: 10.1016/j.indcrop.2018.02.066

Meti S, Meerabai M, Salam M, Jacob J and Vijayaraghavakumar. 2014. Soil nutrient dynamics of mature rubber (*Hevea brasiliensis* Muell. Arg.) plantation in relation to phenology and growing environment. Journal of the Indian Society of Soil Science 62(4): 376-383.

Paranjothy K. 2018. *Hevea*. In: Halevy AH. (ed). Handbook of flowering. CRC Press, inc., Boca Raton. pp. 133-141.

Pohlert T. 2014. The Pairwise Multiple Comparison of Mean Ranks Package (PMCMR). R package. In: The Comprehensive R Archive Network, https://cran.r-project.org/web/packages/PMCMR/ Accessed: January 2019.

Priyadarshan PM. 2017. Biology of *Hevea* rubber. Springer. 251 p. doi: 10.1007/978-3-319-54506-6

R Core Team. 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Richardson AD, Toomey M, Migliavacca M, Sonnentag O, Keenan TF and Ryu Y. 2013. Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. Agricultural and Forest Meteorology 169: 156–173. doi: 10.1016/j.agrformet.2012.09.012

Righi CA, Bernardes MS, Castro DS e Abbud DM. 2001. Fenologia e variação temporal do índice de área foliar de três cultivares de seringueira (*Hevea* spp.). Agrotrópica 13(3): 125 - 132.

Rivano F, Vera J, Cevallos V, Almeida D, Maldonado L and Flori

A. 2016. Performance of 10 *Hevea brasiliensis* clones in Ecuador, under South American Leaf Blight escape conditions. Industrial Crops and Products 94: 762-773. doi: 10.1016/j.indcrop.2016.09.035

Silva JQ, Scaloppi-Júnior EJ, Moreno MB, De Souza, GB, Gonçalves DS y Filho AS. 2012. Producción y propiedades químicas del caucho en clones de *Hevea* según los estados fenológicos. Pesquisa Agropecuária Brasileira 47(8): 1066–1076. doi: 10.1590/S0100-204X2012000800006

Simbo DJ, Van Den Bilcke N and Samson R. 2013. Contribution of corticular photosynthesis to bud development in African baobab (*Adansonia digitata* L.) and Castor bean (*Ricinus communis* L.) seedlings. Environmental and Experimental Botany 95: 15. doi: 10.1016/j.envexpbot.2013.07.002

Sterling A, Martínez-Viuche EJ, Pimentel-Parra GA, Suárez-Córdoba YD, Fonseca-Restrepo JA, and Virguez-Díaz YR. 2019. Dynamics of adaptive responses in growth and resistance of rubber tree clones under South American leaf blight non-escape conditions in the Colombian Amazon. Industrial Crops and Products 141: 111811. doi: 10.1016/j.indcrop.2019.111811

Villa MR, Martínez EG, Cartagena JR, Rodríguez OA and Osorio NW. 2017. Characterization of soils cultivated with rubber in the Colombian Bajo Cauca Antioqueño region. Revista Facultad Nacional de Agronomía Medellín 70(2): 8155-8167. doi: 10.15446/rfna.v70n2.64520

Zhai DL, Yu H, Chen SC, Ranjitkar S and Xu J. 2017. Responses of rubber leaf phenology to climatic variations in Southwest China. International Journal of Biometeorology 63(5): 607-616. doi: 10.1007/s00484-017-1448-4

Research article

http://www.revistas.unal.edu.co/index.php/refame

Revista Facultad Nacional de Agronomía

Antioxidant activity and GC-MS profile of *Conyza bonariensis* L. leaves extract and fractions

UNIVERSIDAD NACIONAL DE COLOMBIA

Actividad antioxidante y perfil CG-EM del extracto y fracciones de hojas de *Conyza bonariensis* L.

doi: 10.15446/rfnam.v73n3.81452

Rafael Viteri Espinoza[1]*, Juan Peñarreta[2], María Quijano-Avilés[1], Ana Barragán Lucas[1], Iván Chóez-Guaranda[1] and Patricia Manzano Santana[1,3]

ABSTRACT

The 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing power (FRAP), and semivolatile compounds of *Conyza bonariensis* L. leave extract and fractions are discussed. A methanolic crude extract was obtained through maceration, and subsequently, *n*-hexane, chloroform, and ethyl acetate fractions were collected using a solvent-solvent partition. Total phenols, flavonoids, and antioxidant activity assays were performed in an ultraviolet-visible (UV-Vis) spectrophotometer, and the results were expressed as Gallic Acid, Quercetin, and Trolox equivalents, respectively. The findings achieved indicate that ethyl acetate fraction showed the highest DPPH radical scavenging capacity (90.69±3.16%) at 500 µg mL^{-1}, and reduced the ferric tripyridyltriazine complex (Fe^{3+}-TPTZ) with values between 19.68 and 2,355.37 mg Trolox equivalent (TE) g^{-1}. It was identified 28 phytoconstituents through Gas chromatography-mass spectrometry (GC·MS). The scavenging activity of ethyl acetate fraction could be correlated mostly to the presence of eugenol, trans-isoeugenol, lucenin-2, methyl salicylate, and syringic acid. This study reveals that the ethyl acetate fraction could be used as a good source of antioxidants for health benefits.

Keywords:
Bioactivity
DPPH
Flavonoid
Fractionation
FRAP
Polyphenol

RESUMEN

El 2,2-difenil-1-picrilhidrazil (DPPH), el poder reductor férrico (FRAP) y los compuestos semivolátiles del extracto y fracciones de Conyza bonariensis L. son discutidos. Se obtuvo un extracto crudo en metanol mediante maceración y posteriormente se recogieron fracciones de n-hexano, cloroformo y acetato de etilo usando partición disolvente-disolvente. Los ensayos de fenoles totales, flavonoides y actividad antioxidante se efectuaron en un espectrofotómetro (UV-Vis) y los resultados se expresaron como equivalentes de Ácido gálico, Quercetina y Trolox, respectivamente. Los resultados indican que la fracción en acetato de etilo mostró la mayor capacidad de eliminación del radical DPPH (90,69±3,16%) a 500 µg mL^{-1} y redujo el complejo de tripiridiltriazina férrico (Fe3+-TPTZ) con valores entre 19,68 y 2355,37 mg equivalente de Trolox (TE) g^{-1}. Se identificaron 28 fitoconstituyentes a través del análisis de cromatografía de gases-espectrometría de masas (CG-EM). La actividad antioxidante presentada puede correlacionarse principalmente con la presencia de eugenol, trans-isoeugenol, lucenina-2, salicilato de metilo y ácido siríngico detectado. Este estudio muestra que la fracción de acetato de etilo podría ser utilizada como una buena fuente de antioxidantes para el beneficio de la salud humana.

Palabras clave:
Bioactividad
DPPH
Flavonoide
Fraccionamiento
FRAP
Polifenoles

[1] Centro de Investigaciones Biotecnológicas del Ecuador. Escuela Superior Politécnica del Litoral. Campus Gustavo Galindo km 30.5 vía Perimetral, Guayaquil, Ecuador.
[2] Facultad de Ciencias Químicas. Universidad de Guayaquil. Cdla. Universitaria Salvador Allende, Malecón del Salado entre Av. Delta y Av. Kennedy. Guayaquil, Ecuador.
[3] Facultad de Ciencias de la Vida. Escuela Superior Politécnica del Litoral. Campus Gustavo Galindo, km. 30.5 vía Perimetral. Guayaquil, Ecuador.
* Corresponding author: <rviterie@gmail.com>

Received: August 2, 2019; Accepted: April 9, 2020
Rev. Fac. Nac. Agron. Medellín 73(3): 9305-9313. 2020
ISSN 0304-2847 / e-ISSN 2248-7026

Conyza bonariensis L. is a short-lived perennial weed native from South America, extensively spread over tropical and warm temperate areas worldwide (Zambrano-Navea *et al*., 2016). This species is part of the Asteraceae family, which is popular in the folk medicine, and large number of species are reported to be rich in phenolic compounds and acidic polysaccharides. Besides, some species have been described to possess biological activities such as antiplatelet, anticoagulant, and antioxidant properties (Saluk-Juszczak *et al*., 2010). *C. bonariensis* is considered as a glyphosate-resistant weed that causes serious problems to productive crops (Okumu *et al*., 2019). Nonetheless, *C. bonariensis* is widely used in traditional medicine for the treatment of rheumatism, gout, and nephritis. Furthermore, antioxidant, antibacterial, and hepatoprotective activities have been reported for *C. bonariensis* extracts (Thabit *et al*., 2015). Natural extracts that exhibit antioxidant and antimicrobial activities represent a promising alternative to chemical products because of their high effectiveness, low cost, and non-environmental pollutants (Rodríguez *et al*., 2000). *C. bonariensis* tinctures have been shown notably inhibitory activity against fungi that cause skin superficial infections, such as dermatophytes, Candida, and Malassezia (Mussin *et al*., 2017). In this context, the exploitation of *C. bonariensis* properties would represent a great benefit for the socio-economic development of countries where the weed interferes with the growth of crops. Even though there is information available about biological activities of *C. bonariensis*, the species that grow in Ecuador has not been studied yet. Due to a lack of information, this study focuses on determining the chemical profile and antioxidant activity of extracts and fractions of *C. bonariensis* leaves extract and its fractions in order to support the beneficial use of this weed in Ecuador.

MATERIALS AND METHODS

Plant material

The leaves of *C. bonariensis* were collected in, Guayaquil (Guayas, Ecuador) in 2014 and authenticated by National Herbarium (Quito, Ecuador). A voucher specimen (no. CIBE002) has been retained at Centro de Investigaciones Biotecnológicas del Ecuador.

Standards and reagents

The chemicals used were of reagent grade or higher. Folin-Ciocalteu, 2,2-diphenyl-1-picrylhydrazy (DPPH), gallic acid, ascorbic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), bis (trimethylsilyl)-trifluoroacetamide, methanol, sodium nitrite, aluminum chloride, and 2,4,6-tripyridyl-s-triazine (TPTZ) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chloroform, *n*-hexane, and ethyl acetate were purchased from Fisher Scientific (Pittsburgh, PA, USA). Iron (III) chloride hexahydrate was purchased from Merck (Darmstadt, Germany), hydrochloric acid from Mallinckrodt (St. Louis, MO, USA), sodium acetate trihydrate and acetic acid from J.T. Baker (Phillipsburg, NJ, USAG). Ethanol was purchased from Panreac (Barcelona, Spain). Ultrapure water was purified in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Extraction procedure

The leaves of *C. bonariensis* were dried at 37 °C for 48 h in a stove; they were pulverized with a hand mill and passed through a mesh sieve of 0.5 mm. Then, 100 g of dried plant material was macerated with methanol (MeOH) for 48 hours for extraction. The crude methanol extract (ME) was obtained by solvent evaporating, using a rotary evaporator to obtain 10.24 g of total extract (yield 12.24%). This extract was solubilized in H_2O:MeOH (300 mL, 1:1) and subsequently partitioned with *n*-hexane (3×150 mL, HF), chloroform (3×150 mL, CF), and ethyl acetate solvents (3×150 mL, EAF). The remaining water part was freeze dried to obtain an aqueous fraction (AqF).

Total phenolic content

Total phenolic content of the fractions was analyzed using a modification of the Folin-Ciocalteu reagent described by Waterhouse (2002). A calibrated method was used according to the equation y=0.0008x-0.0194 (R^2= 0.994) obtained from the standard gallic acid graph. The total phenolic content was expressed as milligram gallic acid equivalents per gram of dry extract or fraction (mgGAE g^{-1}).

Total flavonoid content

Total flavonoid content was estimated using the spectrophotometric method described by Min *et al.* (2011) using quercetin as a standard. The total flavonoid content was expressed as milligram quercetin equivalents per gram of dry extract or fraction (mgQE g^{-1}). The calibration curve was y=0.0005x-0.0009 (R^2=0.9987) obtained from the standard quercetin graph.

DPPH radical scavenging capacity

The DPPH radical scavenging capacity was determined using the method described by Ebada *et al*. (2008) with minor modifications. Each fraction was reconstituted in ethanol to give concentration ranging from 50 to 500 mg L^{-1}. The reconstituted fraction in ethanol (0.10 mL) was mixed with 1.6 mL DPPH solution (40 mg L^{-1} in 100% ethanol). Ethanol and DPPH solution were used as blank and negative control, respectively. The solutions were mixed and incubated in darkness at room temperature for 30 min. The absorbance was measured at 517 nm, and the DPPH radical scavenging capacity (RSC) was calculated according to $RSC=((A_0-A_1)/A_0)\times100$ equation, where A_0 is the absorbance of the control and A_1 is the absorbance of the fraction.

Ferric reducing antioxidant power (FRAP) assay

The reducing power was determined based on the method used by Li *et al*. (2012). The FRAP assay measures the ability of the antioxidants in the vegetable extracts to reduce ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex to the blue colored ferrous form (Fe^{2+}) which absorbs light at 593 nm. FRAP activity were expressed as milligram Trolox equivalents per gram of dry extract/fraction (mgTE g^{-1}). The calibration curve was $y=0.0031x+0.0061$ ($R^2=0.9989$) obtained from the standard Trolox graph**.**

Gas Chromatography-Mass Spectrometry (GC-MS)

Ethyl acetate fraction of *C. bonariensis* was analyzed using a gas chromatography-mass spectrometry equipment Agilent Technologies (7890A GC system and 5975C inert XL MSD with triple-axis detector). The procedure used for this process was adapted from the method described by Balladares *et al*. (2016). A capillary column HP-5MS (30 m×0.25 mm) with phenyl methylpolysiloxane was used as stationary phase (0.25 µm film thickness) and helium as the carrier gas (1.2 mL min^{-1}). Samples dissolved or suspended in a solvent different from the original extraction solvent were filtered or centrifuged to get rid of any insoluble matter. The injection of 2.0 µL of sample (10 mg mL^{-1}) was done at a temperature of 250 °C with splitless mode, the detector temperature was 280 °C and the oven temperature was maintained at 70 °C for 2.0 min, then it was increased up to 285 °C at a rate of 5 °C min^{-1}. The electron ionization was set at 70 eV, 230 °C was used as ion source, and the data compounds were collected with the full scan mode (40-1000 amu). The identification of the components was based on comparison of their retention index using *n*-alkanes (C7–C40) and mass spectra database of Wiley 9th with NIST 2011 MS Library.

Statistical Analysis

Results were expressed as mean±standard deviation of three repetitions. The assays were compared by using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test, with statistical significance determined at the $P<0.05$. All statistical analyses were performed using Minitab 16 software.

RESULTS AND DISCUSSION

Extraction procedure

The maceration process of dried leaf powder (100 g) in methanol showed a yield of 12.80% after solvent evaporation. The liquid-liquid fractionation revealed the following yields: hexane (44.3%), chloroform (13.7%), ethyl acetate (3.0%), and aqueous fraction (37.9%). These results support that alcoholic extracts (MeOH or EtOH) from plant materials contain a wide variety of polar and nonpolar compounds. Several studies have shown that polar solvents are effective for the extraction of polyphenols (Sacchi *et al*., 2005). Furthermore, it has been reported that the antioxidant activity is attributed to the presence of phenolic compounds and flavonoids in these extracts (Figueroa *et al*., 2015). In this sense, the yield of the ME (12.8%) was higher than the previously obtained from *C. bonariensis* by El Zalabani *et al*. (2012) using 70% ethanol (3.89%), and Kong *et al*. (2001) using methanol (6.33%).

Total phenolic content

The total phenolic content of *C. bonariensis* leave extract and fractions ranged from 27.90 to 340.84 mgGAE g^{-1} for HF and EAF, respectively (Table 1). The phenolic content of EAF presented a significant difference in comparison with the remaining fractions ($P<0.05$), except for CF, AqF, and ME ($P>0.05$). Some studies have reported that ethanolic extracts of *C. bonariensis* obtained from the whole plant showed values between 56.6 and 144.1 mgGAE g^{-1}, informed by Diaz *et al*. (2012) and Thabit *et al*. (2015), respectively. Similarly, the research carried out by Daur (2015) showed a total phenolic content of 78.0 mgGAE g^{-1} in the methanolic extract of the complete plant. Also, Shahwar *et al*. (2012) described a higher total phenolic content (241.3 mgGAE g^{-1}) for the methanolic extract of the stem. In fact, total phenolic content of EAF obtained

by liquid fractionation is slightly higher (395.6 mgGAE g^{-1}) than the other investigations reported.

Diaz *et al*. (2012) also reported a high phenolic content (200.0 mgGAE g^{-1}) from an extract in hot water (autoclaved 121 °C) while El Zalabani *et al*. (2012) reported a low phenolic content (0.96 mgGAE g^{-1}). This variability of the total phenolic content can be attributed to the geographic site where the plant grows and to the solvent used for the extraction.

Table 1. Total phenolic and flavonoid content of ME and its fractions.

Sample	Total phenolic content (mgGAE g^{-1})	Total flavonoid content (mgQE g^{-1})
ME	99.75±3.94 ad	241.79±7.34 a
HF	27.90±1.38 b	46.90±1.69 b
CF	118.07±6.45 a	166.01±15.25 a
EAF	340.84±22.82 c	795.11±60.88 c
AqF	78.49±2.49 d	200.48±5.34 a

ME: crude methanol extract; HF: *n*-hexane fraction; CF: chloroform fraction; EAF: ethyl acetate fraction; AqF: aqueous fraction. Different letters indicate significant differences among fractions/extract (Tukey test, $P<0.05$)

Total flavonoid content

The total flavonoid content of *C. bonarienesis* extract and fractions ranged from 46.90 to 795.11 mgQE g^{-1} for HF and EAF, respectively (Table 1). The flavonoid content of the EAF showed a significant difference compared with the remaining fractions ($P<0.05$) except for CF, AqF, and ME ($P>0.05$).

The total flavonoid content obtained was higher than values reported by Diaz *et al*. (2012) (73.4 mgQE g^{-1}) and Thabit *et al*. (2015) (134.0 mgQE g-1) for whole plant extracts in EtOH and EtOH 90%, respectively. In the same study, Diaz *et al*. (2012) also reported a high flavonoid content (276.4 mgQE g^{-1}) from an extract in hot water (autoclaved 121 °C). This study is comparable to the study informed by Kamdem-Boniface and Pal (2013), which reports a flavonoid content of 199.3 mgQE g^{-1} in *C. sumatrensis*, a species from the same family.

Flavonoids are polyphenolic compounds with great importance that act as potent antioxidants depending on their molecular structures. Quercetin is the most abundant flavonoid with antioxidant properties. Hence, the high content of phenolic compounds and flavonoids in the soluble fraction of ethyl acetate could suggest a good source of bioactive compounds with an interest for human health.

DPPH free radical scavenging capacity

The radical scavenging capacity evaluated between a DPPH concentration range of 50-500 µg mL^{-1} showed inhibition percentages from 10.07% (HF) to 90.69% (EAF) (Table 2). Figure 1 shows the screening profiles of the ME and its fractions, where it can be observed a dose-dependent behavior. The EAF showed the highest activity with an IC_{50} value of 146.9 µg mL^{-1}. The results obtained were significantly different ($P<0.05$).

The DPPH scavenging activity of the methanolic extract (47.8%) of the present study was lower than the reported for the ethanolic extract of *C. bonariensis* whole plant at a concentration of 612 µg mL^{-1}. However, the concentration of the DPPH used in our study (0.1 mM) is higher than the concentration (0.0055 mM) used by Diaz *et al*. (2012).

This study corroborates the potent antioxidant action of the EAF compared to the other fractions assessed. This behavior is possibly explained by the high phenols and flavonoids content obtained in this fraction. EAF exhibited antioxidant activity value comparable to the result obtained by Shahwar *et al*. (2012), who showed (90.3%), and IC_{50} of 89.0 µg mL^{-1} for the ethyl acetate fraction with a concentration of 500 µg mL^{-1}. This value is lower than the result obtained in the present study (146.9 µg mL^{-1}). The other fractions obtained an IC_{50}>500 µg mL^{-1}.

Table 2. Activity antioxidant of ME and its fractions.

Sample	Free radical scavenging activity DPPH inhibition (%)	Antioxidant activity FRAP (mgTE g^{-1})
ME	47.83±0.51 b	262.02±7.09 b
HF	10.07±1.50 e	19.68±2.22 b
CF	26.56±3.87 d	182.33±4.75 b
EAF	90.69±3.16 a	2,355.37±255.11 a
AqF	38.81±3.01 c	202.29±4.82 b

ME: crude methanol extract; HF: n-hexane fraction; CF: chloroform fraction; EAF: ethyl acetate fraction; AqF; aqueous fraction. Different letters indicate significant differences between fractions/extract (Tukey test, $P<0.05$)

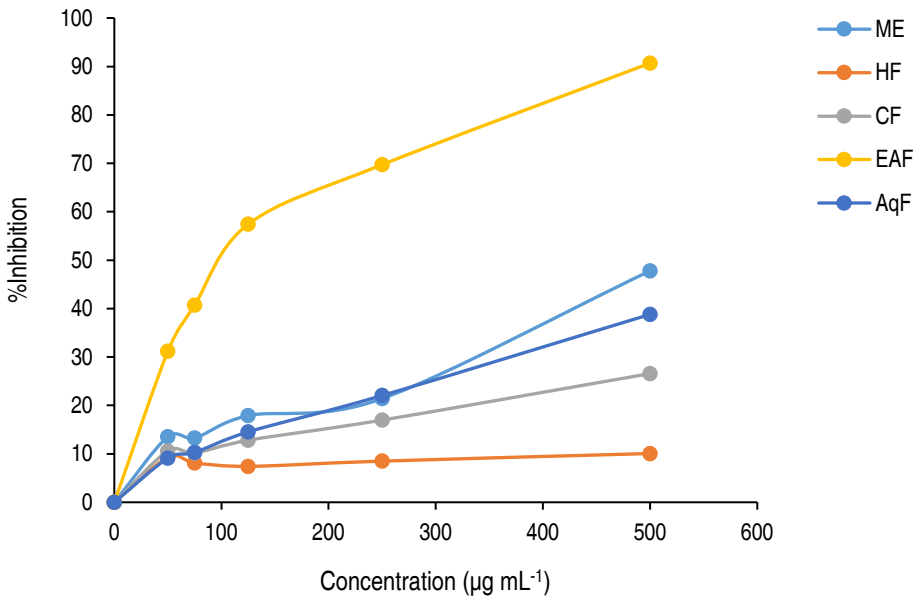


Figure 1. Inhibition effect of ME and its fractions on DPPH

Hayet *et al*. (2009) reported a similar result with an IC_{50} of 150.0 µg mL^{-1} for the ethyl acetate extract of the aerial parts of *Conyza canadensis*, assesed with a 0.075 mM DPPH solution. Furthermore, our results are comparable with the *Ageratum houstonianum* (Asteraceae). In this study, the ethyl acetate extract of the leaves reported a percentage of inhibition of 88.26% with a concentration of 500 µg mL^{-1} (Tennyson *et al*., 2012).

Additionally, there is a positive Pearson correlation between DPPH antioxidant activity, total phenol content, and total flavonoid content (r=0.929 for DPPH and totalphenolic content, r=0.962 for DPPH and total flavonoid content). An extract is considered active when IC_{50} values range from 50 to 100 µg mL^{-1}, and moderate when the values range from 101 to 250 µg mL^{-1} (Marjoni and Zulfisa, 2017).

Ferric reducing antioxidant power (FRAP) assay

In the FRAP assay of *C. bonarienesis* extract and fractions ranged from 19.68 to 2,355.37 mgTE g^{-1} for HF and EAF, respectively (Table 2). EAF gave a high antioxidant power to reduce Fe^{3+} and was significantly different from the remaining fractions ($P<0.05$). Therewas not a significant difference in other fractions ($P>0.05$). Currently, the antioxidant activity

of *Conyza* sp.expressed as Trolox equivalent has not been reported, therefore, it is not possible to make comparisons with the same genus. However, when compared with other plants of the Asteraceae family, the value obtained in *C. bonariensis* ME (1,015.42 mgTE g^{-1}) is higher. For example, Santos *et al.* (2015), reported an antioxidant activity from aqueous extract of *Tagetes erecta* of 24.9 mgTE g^{-1}, hydroethanolic extract of 2.7 mgTE g^{-1}, and the ethanolic extract of 25.9 mgTE g^{-1} while *Tagetes patula* showed values of 22.5 and 21.0 mgTE g^{-1} for hydroethanolic and ethanolic extracts, respectively.

In addition, comparisons with medicinal plants from Turkey, the antioxidant activity of this study was higher than the activity obtained for *Achillea phrygia* (Asteraceae) with ethyl acetate, methanol, and aqueous extract with values of 52.0, 129.9, and 130.6 mgTE g^{-1}, respectively. *Bupleurum croceum* (Apiaceae) showed values of 64.6, 72.6, and 87.9 mgTE g^{-1} for the ethyl acetate, methanol, and aqueous extract, respectively. Ceylan *et al.* (2016) reported lower antioxidant activity of methanolic extract of aerial parts of *Cytisopsis dorycniifolia* and *Ebenus hirsuta*, both members of Fabaceae family, 200.6 and 103.4 mgTE g^{-1}, respectively.

The DPPH and FRAP trials were largely in agreement with each other, which is reflected in the high Pearson correlation (r=0.917) between both trials. Therefore, the antioxidant activity for both DPPH and FRAP methods could be due to the presence of phenolic and flavonoids compound with a high correlation value (r=0.973) for both.

Gas chromatography/mass spectrometry analysis

Based on the high phenolic and flavonoid contents and antioxidant activity obtained in the present study, the EAF was subjected to GC-MS analysis. A total of 102 compounds were detected in EAF, but it was only possible to identify 28 corresponding to 36.59% (Table 3). These compounds were mainly esterified fatty acids, free fatty acids, and alcohols, but also the presence of monosaccharides, phenolic, and flavonoid acids were detected (Figure 2).

Table 3. Compounds identified by GC-MS in *C. bonariensis* ethyl acetate fraction.

Peak	RT (min)	Compounds	RI	RIL	% area
1	8.21	Levoglucosenone	-	1070	3.45
2	9.88	Butanedioic acid, diethyl ester	-	1149	0.10
3	10.31	Benzoic acid, 2-hydroxy-, methyl ester	-	1176	0.18
4	11.02	1,4:3,6-Dianhydro-α-d-glucopyranose	1217.15	1198	2.25
5	13.09	Phenol, 4-ethoxy	1294.25	1330	1.51
6	14.07	2,2-Dimthyl-3-phenyl-2,5-dihydrofuran	1331.33	1368	0.27
7	14.56	Eugenol	1349.92	1337	0.40
8	17.02	Trans-Isoeugenol	1445.91	1429	0.31
9	18.47	Ethyl 3-hydroxybenzoate	1504.41	1380	0.86
10	18.74	Calamenene	1515.82	1517	1.27
11	20.80	Mesityl-3-methyl-1-butanol	1602.27	1629	0.87
12	21.40	Blumenol C	1629.15	1713	0.90
13	25.59	Syringic acid	1821.72	1813	1.23
14	25.88	p-Hydroxycinnamic acid, ethyl ester	1835.83	1887	0.64
15	27.48	Ethyl ferulate	1914.43	1776	3.19
16	27.95	Oleic Acid	1938.38	2113	0.15
17	28.44	Palmitic acid	1963.34	1942	2.66

Continuation Table 3

Peak	RT (min)	Compounds	RI	RIL	% area
18	28.91	Palmitic acid, ethyl ester	1987.09	1968	2.79
19	29.92	Myristyl monoethoxylate	2040.61	1930	1.54
20	31.53	8-Heptylpentadecane	2127.99	2144	2.13
21	31.97	Ethyl linoleate	2152.42	2193	0.26
22	32.10	Ethyl Oleate	2159.60	2185	3.89
23	32.22	Oleic Acid	2166.33	2133	2.90
24	32.60	Stearic acid, ethyl ester	2187.59	2181	1.90
25	34.93	Isochiapin B	2324.07	2577	0.41
26	35.55	9-Octadecenamide	2361.58	2333	2.23
27	35.73	1-Monopalmitin	2372.01	2482	0.91
28	44.39	Lucenin 2	2961.41	2499	0.20
Total					**36.59**

RT: retention time; RI: retention index relative to a standard mixture of *n*-alkanes on the HP-5MS column; RIL: retention index library.

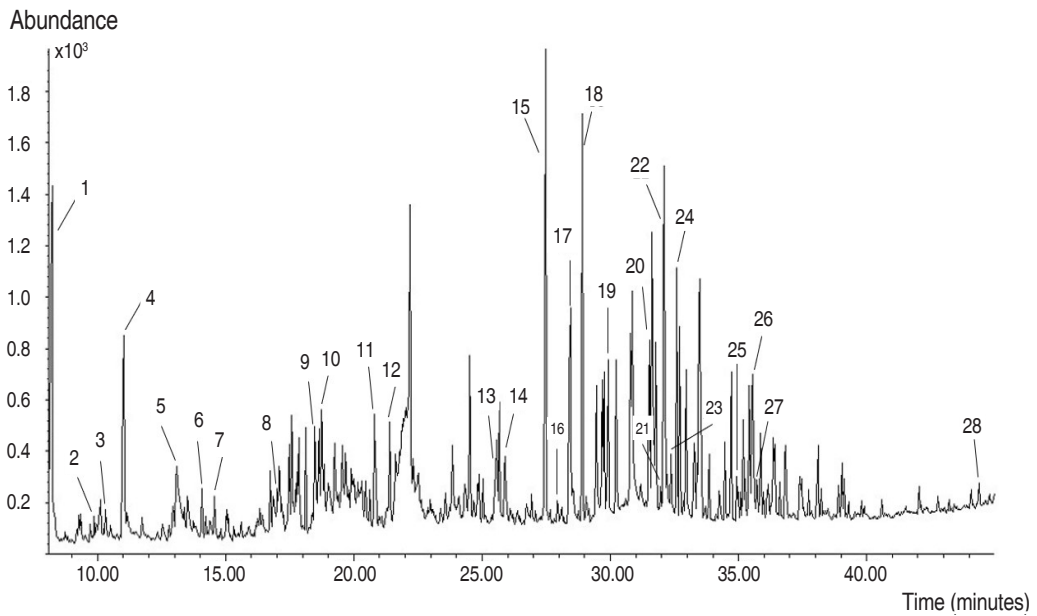


Figure 2. Chromatogram of the GC-MS analysis of *C. bonariensis* ethyl acetate fraction.

Antioxidant activity of ethyl acetate fraction of *C. bonariensis* could be explained by the presence of the flavonoid lucenin-2, methyl salicylate, the aromatic compound eugenol, trans-isoeugenol, and the sesquiterpene lactone isochiapin B due to phenyls groups and double bonds in their chemical structures able to stabilize free radicals (Choe and Min, 2009). Lucenin-2 has been reported as remarkable antihepatotoxic activity against CCl_4 and galactosamine cytotoxicity in primary cultured rat hepatocytes (Hoffmann-Bohm *et al.*, 1992). Besides this *c*-glycosyl compound has been isolated from the leaves of *Passiflora edulis* f. *flavicarpa*, contributing to the positive anxiolytic activity of leaf ethanol extracts evaluated, and it has been shown to

induce dose-dependent alterations on germination and growth of *Tortula muralis* increasing spore germination, protonemal length, seed germination, and length of ipocothyl-root until 18 days of inoculation (Basile *et al*., 2003). Several biological activities have been attributed to eugenol such as anticonvulsant, analgesic, anti-inflammatory, antifungal, antioxidant, and radical-scavenging activity (Singh and Chaudhuri, 2018). Furthermore, levoglucosenone has been demonstrated to have antitumor activity (Giri *et al*., 2016). Besides, syringic acid has exhibited anti-inflammatory, antioxidant, antimicrobial, anticancer properties (Kumar *et al*., 2012). On the other hand, the sesquiterpene lactone isochiapin B has been used to treat arthritis, tonsillitis, and other ailments by Chinese medicine (Krishnan and Murugan, 2014). It is important to note that sesquiterpene lactones are characteristic secondary metabolites of the Asteraceae family derivative of sesquiterpenoids which have been shown to possess antimalarial, antimicrobial, antitumor, anti-inflammatory and antioxidant activity (Shoaib *et al*., 2017).

CONCLUSIONS

This study reports that ethyl acetate fraction of *C. bonariensis* leaves showed a relevant antioxidant activity, which could be correlated to the presence of eugenol, trans-isoeugenol, lucenin-2, methyl salicylate, and syringic acid. These results confirm that this plant species is a good source of antioxidants that could be medicinally useful.

ACKNOWLEDGMENTS

This work was financed by Escuela Superior Politécnica del Litoral (ESPOL).

REFERENCES

Balladares C, Chóez-Guaranda I, García J, Sosa D, Pérez S, González JE, Viteri R, Barragán A, Quijano-Avilés M and Manzano P. 2016. Physicochemical characterization of *Theobroma cacao* L. sweatings in Ecuadorian coast. Emirates Journal of Food and Agriculture 28(10): 741-745. doi: 10.9755/ejfa.2016-02-187

Basile A, Sorbo S, López-Sáez JA, and Castaldo Cobianchi R. 2003. Effects of seven pure flavonoids from mosses on germination and growth of *Tortula muralis* HEDW. (Bryophyta) and *Raphanus sativus* L. (Magnoliophyta). Phytochemistry. 62(7): 1145–1151. doi: 10.1016/S0031-9422(02)00659-3

Ceylan R, Katanić J, Zengin G, Matić S, Aktumsek A, Boroja T, Stanić S, Mihailović V, Guler GO, Boga M and Yılmaz MA. 2016. Chemical and biological fingerprints of two Fabaceae species (*Cytisopsis dorycniifolia* and *Ebenus hirsuta*): Are they novel sources of natural agents for pharmaceutical and food formulations? Industrial Crops and Products 84: 254–262. doi: 10.1016/j.indcrop.2016.02.019

Choe E and Min DB. 2009. Mechanisms of Antioxidants in the Oxidation of Foods. Comprehensive Reviews in Food Science and Food Safety 8(4): 345–358. doi: 10.1111/j.1541-4337.2009.00085.x

Daur I. 2015. Chemical composition of selected Saudi medicinal plants. Arabian Journal of Chemistry 8(3): 329–332. doi: 10.1016/j.arabjc.2013.10.015

Diaz P, Jeong SC, Lee S, Khoo C and Koyyalamudi SR. 2012. Antioxidant and anti-inflammatory activities of selected medicinal plants and fungi containing phenolic and flavonoid compounds. Chinese Medicine. 7(1): 26. doi: 10.1186/1749-8546-7-26

Ebada SS, Edrada RA, Lin W and Proksch P. 2008. Methods for isolation, purification and structural elucidation of bioactive secondary metabolites from marine invertebrates. Nature Protocols 3(12): 1820–1831. doi: 10.1038/nprot.2008.182

El Zalabani SM, Hetta MH and Ismail AS. 2012. Genetic profiling, chemical characterization and biological evaluation of two *Conyza* species Growing in Egypt. Journal of Applied Pharmaceutical Science 2(11): 54–61. doi: 10.7324/JAPS.2012.21110

Figueroa L, Navarro L, Vera M and Petricevich V. 2015. Antioxidant activity, total phenolic and flavonoid contents, and cytotoxicity evaluation of *Bougainvillea xbuttiana*. International Journal of Pharmacy and Pharmaceutical Sciences 6(5): 497–502.

Giri GF, Danielli M, Marinelli RA and Spanevello RA. 2016. Cytotoxic effect of levoglucosenone and related derivatives against human hepatocarcinoma cell lines. Biorganic & Medicinal Chemistry Letters 26(16): 3955–3957. doi: 10.1016/j.bmcl.2016.07.007

Hayet E, Maha M, Samia A, Ali MM, Souhir B, Abderaouf K, Mighri Z and Mahjoub A. 2009. Antibacterial, antioxidant and cytotoxic activities of extracts of *Conyza canadensis* (L.) Cronquist growing in Tunisia. Medicinal Chemistry Research 18(6): 447–454. doi: 10.1007/s00044-008-9141-0

Hoffmann-Bohm K, Lotter H, Seligmann O and Wagner H. 1992. Antihepatotoxic *C*-glycosylflavones from the leaves of *Allophyllus edulis* var. *edulis* and *gracilis*. Planta Medica 58(6): 544–548. doi: 10.1055/s-2006-961546

Kamdem-Boniface P and Pal A. 2013. Substantiation of the ethnopharmacological use of *Conyza sumatrensis* (Retz.) E.H.Walker in the treatment of malaria through *in vivo* evaluation in *Plasmodium berghei* infected mice. Journal of Ethnopharmacology 145(1): 373–377. doi: 10.1016/j.jep.2012.10.025

Kong LD, Abliz Z, Zhou CX, Li LJ, Cheng CHK and Tan RX. 2001. Glycosides and xanthine oxidase inhibitors from *Conyza bonariensis*. Phytochemistry 58(4): 645–651. doi: 10.1016/S0031-9422(01)00176-5

Krishnan R and Murugan K. 2014. Comparison of GC-MS analysis of phytochemicals in the ethanolic extracts of *Marchantia linearis* Lehm & Lindenb. and *Marchantia polymorpha* L. (Bryophyta). International Journal of Pharmaceutical Sciences and Research. 49(5): 1981–1987. doi: 10.13040/IJPSR.0975-8232.5(5).1981-87

Kumar S, Prahalathan P and Raja B. 2012. Syringic acid ameliorates l-NAME-induced hypertension by reducing oxidative stress. Naunyn-Schmiedeberg's Archives of Pharmacology 385(12): 1175–1184. doi: 10.1007/s00210-012-0802-7

Li H, Deng Z, Zhu H, Hu C, Liu R, Young JC and Tsao R. 2012.

Highly pigmented vegetables: Anthocyanin compositions and their role in antioxidant activities. Food Research International 46(1): 250–259. doi: 10.1016/j.foodres.2011.12.014

Marjoni MR and Zulfisa A. 2017. Antioxidant activity of methanol extract/fractions of senggani leaves (*Melastoma candidum* D. Don). Pharmaceutica Analytica Acta 8(8). doi: 10.4172/2153-2435.1000557

Min B, McClung AM and Chen MH. 2011. Phytochemicals and Antioxidant capacities in rice brans of different color. Journal of Food Science 76(1): C117–C126. doi: 10.1111/j.1750- 3841.2010.01929.x

Mussin JE, Manzano P, Mangiaterra M and Giusiano G. 2017. Inhibitory activity of *Conyza bonariensis* (L.) Cronquist tincture against fungi and bacteria causing superficial infections. Revista Cubana de Plantas Medicinales 22(3).

Okumu MN, Vorster BJ and Reinhardt CF. 2019. Growthstage and temperature influence glyphosate resistance in *Conyza bonariensis* (L.) Cronquist. South African Journal of Botany 121: 248–256. doi: 10.1016/j.sajb.2018.10.034

Rodríguez AT, Morales D and Ramírez MA. 2000. Efecto de extractos vegetales sobre el crecimiento *in vitro* de hongos fitopatógenos. Cultivos Tropicales. 21(2): 79–82.

Sacchi KL, Bisson LF and Adams DO. 2005. A review of the effect of winemaking techniques on phenolic extraction in red wines. American Journal of Enology and Viticulture 56(3): 197–206.

Saluk-Juszczak J, Pawlaczyk I, Olas B, Kołodziejczyk J, Ponczek M, Nowak P, Tsirigotis-Wołoszczak M, Wachowicz B and Gancarz R. 2010. The effect of polyphenolic-polysaccharide conjugates from selected medicinal plants of Asteraceae family on the peroxynitriteinduced changes in blood platelet proteins. International Journal of Biological Macromolecules 47(5): 700–705. doi: 10.1016/j. ijbiomac.2010.09.007

Santos PC, Santos VHM, Mecina GF, Andrade AR, Fegueiredo PA, Moraes VMO, Silva LP and Silva RMG. 2015. Phytotoxicity of *Tagetes erecta* L. and *Tagetes patula* L. on plant germination and growth. South African Journal of Botany 100: 114–121. doi: 10.1016/j.sajb.2015.05.013

Shahwar D, Raza MA, Saeed A, Riasat M, Chattha FI, Javaid M, Ullah S and Ullah S. 2012. Antioxidant potential of the extracts of *Putranjiva roxburghii*, *Conyza bonariensis*, *Woodfordia fruiticosa* and *Senecio chrysanthemoids*. African Journal of Biotechnology 11(18): 4288–4295. doi: 10.5897/AJB11.2564

Shoaib M, Shah I, Ali N, Adhikari A, Tahir MN, Shah SWA, Ishtiaq S, Khan J, Khan S and Umer MN. 2017. Sesquiterpene lactone; a promising antioxidant, anticancer and moderate antinociceptive agent from Artemisia macrocephala jacquem. BMC Complementary and Alternative Medicine 17(27): 1–11. doi: 10.1186/s12906-016-1517-y

Singh D and Chaudhuri PK. 2018. A review on phytochemical and pharmacological properties of Holy basil (*Ocimum sanctum* L.). Industrial Crops and Products 118: 367–382. doi: 10.1016/j. indcrop.2018.03.048

Tennyson S, Balaraju K, Park K, Ravindran KJ, Eapen A and William SJ. 2012. *In vitro* antioxidant activity of *Ageratum houstonianum* Mill. (Asteraceae). Asian Pacific Journal of Tropical Disease 2 Suppl. 2: S712–S714. doi: 10.1016/S2222-1808(12)60249-7

Thabit RA, Cheng XR, Tang X, Sun J, Shi YH and Le GW. 2015. Antioxidant and antibacterial activities of extracts from *Conyza bonariensis* growing in Yemen. Pakistan Journal of Pharmaceutical Sciences. 28(1): 129–134.

Waterhouse AL. 2002. Determination of Total Phenolics. Current Protocols in Food Analytical Chemistry 6(1):1-8. doi: 10.1002/0471142913.fai0101s06

Zambrano-Navea CL, Bastida F and González-Andújar JL. 2016. A cohort-based stochastic model of the population dynamic and long-term management of *Conyza bonariensis* in fruiting tree crops. Crop Protection 80(6): 15–20. doi: 10.1016/j. cropro.2015.10.023

Research article

http://www.revistas.unal.edu.co/index.php/refame

Revista Facultad Nacional de Agronomía

Larvicidal activity of ethanolic extract of *Azadirachta indica* against *Aedes aegypti* larvae

Actividad larvicida del extracto etanólico de *Azadirachta indica* contra larvas de *Aedes aegypti*

UNIVERSIDAD NACIONAL DE COLOMBIA

doi: 10.15446/rfnam.v73n3.80501

Patricia Manzano[1,2,7*], Oscar Valmaña García[3], Jessica Malusín[4], Jenny Villamar[4], María Quijano[1], Rafael Viteri[1], Ana Barragán[1] and Andrea Orellana-Manzano[2,5,6]

ABSTRACT

Aedes aegypti is a mosquito that carries dengue virus, yellow fever and other diseases transmitted to humans. Organophosphorus larvicides are used to control the proliferation of this mosquito, which has generated a high degree of resistance; hence, new alternatives such as bio-larvicides formulated with plant extracts are of great interest. The aims of this study were to evaluate the ethanolic extract of *Azadirachta indica* leaves as a larvicide against *Aedes aegypti* and to determine the main compounds present in it by GC-MS. In the assay, three concentrations of ethanolic extract were used (10 mg L^{-1}, 20 mg L^{-1}, and 50 mg L^{-1}). This was performed thrice against a positive control (commercial larvicide: spores and endotoxic crystals of *Bacillus thuringiensis* var. israelensis Serotype H-14) and negative control (water). After 72 h of incubation, it was observed higher larval mortality (93%) in the ethanolic extract at a concentration of 50 mg L^{-1}; the extracts at 10 mg L^{-1} and 20 mg L^{-1} shown larval mortality of 47% and 70%, respectively. The majority compound determined by the GC-MS analysis was phytol (14.4% area). The results obtained in this study demonstrated the larvicidal potential of the ethanolic extract of *A. indica* against larvae of *A. aegypti*.

Keywords:
Bio-larvicide
Crude ethanolic extract
GC-MS
Mosquito
Neem
Phytol

RESUMEN

Aedes aegypti es un mosquito portador del virus del dengue, la fiebre amarilla y otras enfermedades transmitidas a los humanos. Los larvicidas organofosforados se utilizan para controlar la proliferación de este mosquito, el cual ha generado un alto grado de resistencia, por lo que las nuevas alternativas de biolarvicidas formulados con extractos de plantas son de gran interés. Los objetivos de este estudio fueron evaluar el extracto etanólico de hojas de *Azadirachta indica* como larvicida contra *Aedes aegypti* e identificar los principales compuestos químicos GC-MS. En el ensayo, se utilizaron tres concentraciones de extracto de etanol (10 mg L^{-1}, 20 mg L^{-1} y 50 mg L^{-1}), este se realizó por triplicado contra un control positivo (larvicida comercial: Esporas y cristales endotóxicos de *Bacillus thuringiensis* var. israelensis Serotipo H-14) y un control negativo (agua). Después de 72 horas de incubación, se observó una mayor mortalidad larval (93%) en el extracto etanólico a una concentración de 50 mg L^{-1}, y los extractos a 10 mg L^{-1} y 20 mg L^{-1} mostraron una mortalidad larval del 47% y 70% respectivamente. El compuesto con mayor abundancia determinado por el análisis CG-MS fue el fitol (área del 14,4%). Los resultados obtenidos en este estudio demostraron el potencial larvicida del extracto de etanol de *A. indica* contra las larvas de *A. aegypti*.

Palabras clave:
Biolarvicida
Extracto crudo de etanol
GC-MS
Mosquito
Neem
Fitol

[1] Centro de Investigaciones Biotecnológicas del Ecuador. Escuela Superior Politécnica del Litoral. Km 30.5 Vía Perimetral P.O. Box 09-01-5863, Guayaquil, Ecuador.
[2] Facultad de Ciencias de la Vida. Escuela Superior Politécnica del Litoral. Km. 30.5 vía Perimetral. P.O. Box 09-01-5863, Guayaquil, Ecuador.
[3] Facultad de Ingeniería Química. Universidad de Guayaquil. Av. Delta y Av. Kennedy. P.O. Box 09-06-13, Guayaquil, Ecuador.
[4] Facultad de Ciencias Químicas. Universidad de Guayaquil. Av. Delta y Av. Kennedy, P.O. Box 09-06-13, Guayaquil, Ecuador.
[5] Laboratory for biomedical research. Facultad de Ciencias de la Vida. Escuela Superior Politécnica del Litoral. Km. 30.5 vía Perimetral. P.O. Box 09-01-5863, Guayaquil, Ecuador.
[6] Escuela de Odontología. Universidad Espíritu Santos. Samborondón, Guayas, Ecuador.
[7] Facultad de Ciencias Naturales y Matemáticas. Escuela Superior Politécnica del Litoral. Km. 30.5 vía Perimetral. P.O. Box 09-01-5863, Guayaquil, Ecuador
* Corresponding author:<pmanzano@espol.edu.ec>

Received: November 18, 2019; Accepted: July 9, 2020
Rev. Fac. Nac. Agron. Medellín 73(3): 9315-9320. 2020

ISSN 0304-2847 / e-ISSN 2248-7026

Aedes aegypti, known as mosquito, is widely distributed in America, except Canada and Chile. It is also the vector of several diseases transmitted to humans, including dengue, malaria, and, more recently, chikungunya and zika. Vector-borne diseases are the second group of pathologies with the highest morbidity in Ecuador, led by dengue and chikungunya (Ministerio de Salud Pública, 2015).

Dengue is caused by a virus of the Flaviviridae family, which presents different epidemiological patterns associated with four serotypes that cause this disease (DENV-1, DENV-2, DENV-3, and DENV-4) (WHO, 2020b). It has an alarming impact on human health worldwide due to its easy transportation from one place to another by infected travelers. In 2019, there were more than 3.1 million cases from which 28,000 were severe, and 1,534 ended up in death; currently, around 500 million people are at risk of contracting dengue in Latin America (PAHO, 2020). The WHO has reported several compounds to improve mosquito larvicides, among them are chemical and synthetic organic oils (WHO, 2009). Chemical and biological larvicides are insecticides widely used to kill insects as *A. aegypti* in the larval life stage and to control the pests. Synthetic organophosphate insecticides (temephos and malathion), and pyrethroids (deltamethrin, lambda-cyhalothrin, and cypermethrin) are used for the control of adults of *Aedes aegypti*, especially in the emerging stage; however, the pyrethroid and cypermethrin have become ineffective (Vargas-Miranda *et al*., 2019). Recently, it has been reported that *A. aegypti* has developed resistance to one of the four classes of insecticides most commonly used for its control (pyrethroids, organochlorines, carbamates, and organophosphates) (PAHO, 2020), and 26 countries have detected resistance to all four classes (WHO, 2020a).

Concerns about environmental pollution and the development of insect resistance to chemical larvicides have stimulated the search for natural insecticides derived from plants (Howard *et al*., 2009). Larvicides from botanical origin have increased the general interest, like the ones derived from *Azadirachta indica* (Meliaceae family, commonly known as neem tree), whose insecticidal, pesticidal and larvicidal extracts have been widely studied (Vietmeyer, 1992).

The neem tree is considered one of the most versatile trees in the world, thanks to its ability to grow in areas that reach high temperatures. Its extract composition is considered one of the richest and most complete because of the presence of a great variety of alkaloids, flavonoids, phenolic compounds, steroids and ketones (Imam *et al*., 2012). These compounds can be extracted from the entire tree, seeds, stem, flowers and fruits, varying in concentration depending on the region and the time of year the tree is collected (Fernandes *et al*., 2019). Extracts of *Azadirachta indica* mixed with other plant extracts and synthetic products have been evaluated against different mosquitoes, such as *Cx. quinquefasciatus*, *Cx. pipiens*, *Ae. aegypti, Ae. togoi, and An. stephensi;* showing synergistic, additive and antagonistic effects (Shaalan *et al*., 2005).

Schneider *et al*. (2017) reported the high potential of neem oil to control pupae and adults of *D. saccharalis* present in sugarcane. The study conducted by Lin *et al*. (2016) provided insight into the gene expression of *Monochamus alternatus* (vector of the destructive forest pest pinewood nematode) at the transcriptional level when subjected to azadirachtin, an active compound of neem, confirming its potential against the pest. This enhances the value of azadirachtin as a potential insecticide of natural origin. Besides, the neem extract is considered a growth regulator insecticide for the control of the lesser mealworm beetle *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) (Zorzetti *et al*., 2015). In other applications, Forim *et al.* (2013) developed a method to prepare nanoparticles loaded with neem (*A. indica*) extracts, which presented a promissory larvicidal activity against *Plutella xylostella* with 100% larval mortality.

Azadirachtin's terpenoids and limonoids are the chemicals responsible for the insecticide, larvicide and antibacterial activity, as was reported by Su and Mulla (2003), Ndione *et al*. (2007) and Liu *et al*. (2014). The insecticide activity by the limonoids in azadirachtin is related to the direct inhibition of chitin synthesis and changes of pupation and metamorphosis related to the hormone ecdysone (Wandscheer *et al*., 2004).

Considering the above mentioned, the present study aimed to evaluate the ethanolic extracts of *Azadirachta indica* leaves, which grows in the Ecuadorian coastal zone, against *Aedes aegypti* larvae and to identify the compounds present in the extract responsible for the larvicidal activity.

MATERIALS AND METHODS

Plant material

Leaves of *A. indica* from Guayaquil – Ecuador (2°07'32" S, 79°50'48" W) were collected in February 2015, during the tree flowering stage. A sample of the plant material was taken for botanical identification, being herborized in the National Herbarium of Ecuador (QCNE), keeping an herbal control (Code: CIBE019) in the Bioproducts laboratory of CIBE-ESPOL, Guayaquil, Ecuador.

Plant extract

The plant extract was obtained according to the procedure described by Maragathavalli *et al.* (2012). Fresh leaves were dried in an oven with air recirculation for 24 h at 55 °C for subsequent manual milling and sieving, the selected fraction was the one remaining on the 2-mm mesh sieve. Three successive macerations were performed with ethanol at 96% (100 g dry sample per 500 mL of solvent) under stirring at room temperature for 48 h and then filtered. The solvent of the combined maceration was removed by roto-evaporation at 40 °C, and the extracted material was kept at 4 °C until use (Heidolph, 40001). Three samples (100, 200, 300 mg) were dissolved on 100 mL of distilled water, giving an initial concentration of 1, 2, and 5 mg mL^{-1}.

GC-MS analysis

The plant extract was analyzed by GC-MS according to the method of Umar *et al.* (2014) with some modifications, using Agilent Technologies gas chromatography and mass spectrometry equipment (7890A GC and 5975C XL MSD inert with triple-axis detector). The samples dissolved in distilled water were filtered or centrifuged to remove any insoluble matter. The injection of 2.0 µL of a sample (10 mg mL^{-1}) was performed at 250 °C with no division mode; the detector temperature was 280 °C, and the oven was equipped with an HP-5 capillary column (30 m×0.25 mm ID×0.25 µm film thickness). The GC-MS was programmed with a ramp of 70 °C for 2 min, at a speed of 5 °C min^{-1} until reaching 285 °C. The carrier flow of helium was adjusted at a speed of 1.2 mL min^{-1} to have a run of 45 min. Electronic ionization was set at 70 eV (135 and 230 °C), and the data were collected in full scan mode (40-1000 amu). Lastly, the compounds were identified by comparing their retention index and the Wiley 9th mass spectrum data with the NIST 2011 MS Library.

Larvicidal assay

The Chemistry Faculty of Universidad de Guayaquil provided the *A. aegypti* larvae. The assay was developed according to the methodology of Wandscheer *et al.* (2004) and Cruz-Estrada *et al.* (2013). Three concentrations of neem extract were prepared by diluting 1 mL of the initial extracts (1, 2, and 5 mg mL^{-1}) in 100 mL of water, obtaining final doses of 10 mg L^{-1}, 20 mg L^{-1}, and 50 mg L^{-1}; dose range was selected based on Wandscheer *et al.* (2004). Ten larvae (III and IV instar stage) were placed in each polyethylene plastic containers with test solutions (100 mL) at a temperature from 25 to 30 °C and 12 h photoperiod. The tests were performed thrice. Negative control (NC) was water, and positive control (PC) was Bactivec (Labiofam), bio-larvicide which active ingredient (0.6%) is *Bacillus thuringiensis* var. israelensis; for the assay, it was diluted to the recommended application (1% v/v). After 24, 48, and 72 h, the percentage of mortality was registered.

Statistical analysis

Assumptions of normality and homogeneity of variances were corroborated, then the data was submitted to 2 way ANOVA. Tukey's test at a 5% probability was used for the comparison of means. The statistical software use was MINITAB 16.

RESULTS AND DISCUSSION

In the comparative study of the percentage of mortality (%) of each extract vs. the exposure time, the mortality was proportional to the exposure time ($P<0.05$). Among the different concentrations, significant differences were found ($P<0.05$), by comparing each independent extract, differences between extracts 10 mg L^{-1} and 20 mg L^{-1}, 10 mg L^{-1} and 50 mg L^{-1}, 20 mg L^{-1} and 50 mg L^{-1} were demonstrated ($P<0.05$), indicating that a higher concentration greater the mortality over time, observing a dose-dependent behavior. The extract 50 mg L^{-1} exhibited the highest mortality with values close to the positive control. The mortality percentages of positive control were 50%, 80%, and 100 % at 24, 48, and 72 h, respectively. The negative control had no larvicidal effect, ensuring the results of this study (Figure 1).

The results of this research demonstrate the larvicidal action of neem extract on larvae of *A. aegypti*. The best larvicidal activity (mortality 93%) was obtained at 50 mg L^{-1} of the extract at 72 h. Nour *et al.* (2012) reported the larvicidal

activity against *Aedes aegypti* mosquitoes' larvae of extracts from different parts of *A. indica* (leaves, stem, root, and seed), being the leaves the ones that produced the greatest activity. In their study, they reported a larvicidal activity between 60-70% in 48 h using a concentration of 50 mg L^{-1} of ethanolic *A. indica* leaf extract, similar to those reported in this study. However, here, the result was superior compared to the ethanolic extract obtained by reflux (50% mortality).

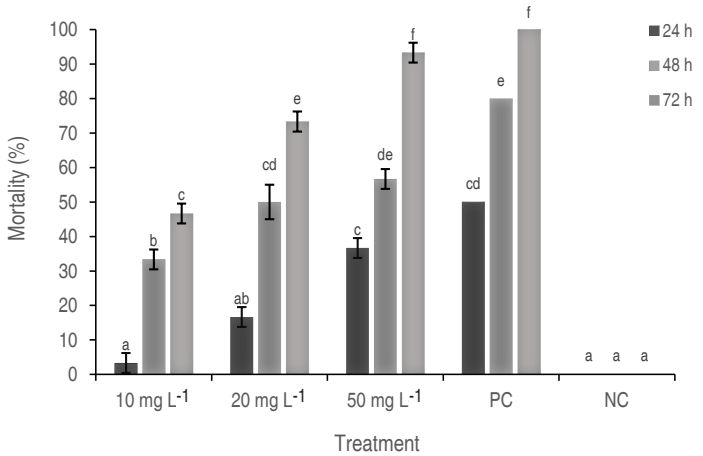


Figure 1. Larval mortality (%) of *A. aegypti* treated with the ethanolic extract of *A. indica* at different concentrations 10 mg L^{-1}, 20 mg L^{-1}, and 50 mg L^{-1}, and Positve (PC, Bactivec) and Negative (NC, water) Control, observed at 24, 48 and 72 h of exposure. Different lowercase letters indicate significant differences among the concentrations by the Tukey test (P<0.05).

Previous research of larvicidal action of ethanolic extract of *A. indica* seeds against *Aedes aegypti* was reported by Wandscheer *et al.* (2004), obtained an LC_{50} value of 440 mg L^{-1}. On the other hand, Shaima *et al.* (2006) reported a larvicidal activity of the methanolic *A. indica* leaf extract against *Anopheles stephensi* with LC_{50} values of 18.2 and 13.1 ppm, after 24 and 48 h, respectively. This demonstrates the larvicidal potential of *A. indica* extract for different species of mosquitoes.

Differences between larvicidal activities could be attributed to the different methods of drying employed. The oven with recirculating air used in this research could cause thermal degradation of the active compounds present in the leaves, reducing the larvicidal activity of extract compared to those reported by Maragathavalli *et al.* (2012) for the same species in another geographical ecological environment.

GC-MS analysis

In Figure 2, the gas analytical chromatogram of the compounds identified by GC-MS of the ethanolic extract is shown. The significant component detected in the extract was phytol (14.24%). The presence of terpenes and fatty acids was also reported (Table 1).

The presence of the terpene phytol, as the main component of the ethanolic extract of *A. indica* is consistent with that reported by Cruz-Estrada *et al.* (2013). Furthermore, previous reports suggest that phytol extracted from plants have larvicidal activity against mosquitoes (Renjana and Thoppil, 2013). Phytol presented in the leaf extract of *Lantara chamber*, *Azadirachta indica* and *Ocimum gratissimum* possess larvicidal properties against *Aedes aegypti* and *Culex quinquefasciatus* (Maneemegalai and Sathish, 2008; Maragathavalli *et al.*, 2012; Pratheeba *et al.*, 2015), and *Premna latifolia* extract against *Aedes albopictus* as observed in the studies conducted by Krishnaveni and Ramamurthy (2014). The combination of neophytadiene and phytol has been shown to have higher insecticidal activity than its components alone (Cáceres *et al.*, 2015). Additionally, potent toxicity of 2-Furancarboxaldehyde has been shown against *Drosophila melanogaster* larvae (Miyazawa *et al.*, 2003). On the other hand, linoleic acid has larvicidal activity against *Aedes aegypti* with LC_{50} and LC_{90} values of 35.39 and 96.33 ppm, respectively (Rahuman *et al.*, 2008), so this compound in the present study could be contributing to larvicidal activity.

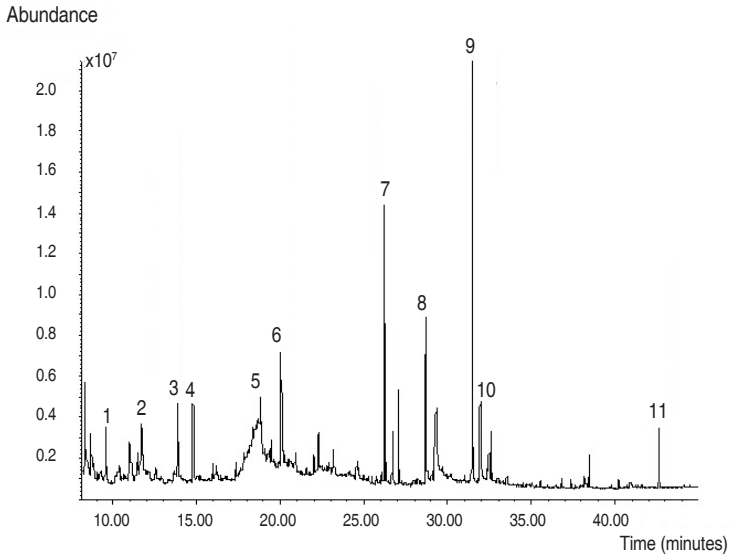


Figure 2. Chromatogram of the ethanolic extract of the leaves of *Azadirachta indica*.

Table 1. Chemical Compounds identified by GC-MS in the ethanolic extract of *Azadirachta indica* leaves.

Peak	Retention Time (min)	Relative abundance (%)	Name of compound
1	9.543	2.53	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one
2	11.675	6.29	2-Furancarboxaldehyde
3	13.866	4.58	2-Methoxy-4-vinylphenol
4	14.787	3.22	Syringol (Ether)
5	18.814	4.87	Phenol, 2,4-bis(1,1-dimethylethyl)
6	20.054	2.93	2,6-Dimethyl-3-(methoxymethyl)-p-benzoquinone
7	26.205	8.10	Neophytadiene
8	28.701	7.23	Palmitic acid
9	31.465	14.24	Phytol
10	31.955	4.07	Linolenic acid
11	42.630	2.02	Squalene oil

According to the results mentioned above may be noted that the neem tree leaves have a high amount of terpenes, which is corroborated by this study where phytol was the most abundant metabolite, a component that confers the insecticide property and possibly the responsible of the reported larvicidal action.

CONCLUSIONS

In this research, it was observed higher larval mortality (93%) after 72 h incubation in the ethanolic extract at a concentration of 50 mg L^{-1}, compared with the extracts at 10 mg L^{-1} and 20 mg L^{-1} shown larval mortality of 47% and 70%, respectively. In the analysis by GC-MS, the presence of phytol was evident as a majority component in the ethanolic extract (14.24%).

ACKNOWLEDGEMENTS

The authors thank the Escuela Superior Politecnica del Litoral (ESPOL) for the financial support to perform this study, and Dr. Maria C. Villacrés, PROMETEO-SENESCYT, for helping in the correction of the manuscript.

REFERENCES

Cáceres LA, McGarvey BD, Briens C, Berruti F, Yeung KKC and Scott IM, 2015. Insecticidal properties of pyrolysis bio-oil from greenhouse tomato residue biomass. Journal of Analytical and Applied Pyrolysis 112: 333-340. doi: 10.1016/j.jaap.2015.01.003

Cruz-Estrada A, Gamboa-Angulo M, Borges-Argáez R and Ruiz-Sánchez E. 2013. Insecticidal effects of plant extracts on immature whitefly *Bemisia tabaci* Genn. (Hemiptera: *Aleyroideae*). Electronic Journal of Biotechnology 16(1): 6. doi: 10.2225/vol16-issue1-fulltext-6

Fernandes SR, Barreiros L, Oliveira RF, Cruz A, Prudêncio C, Oliveira AI, Pinho C, Santos N and Morgado J. 2019. Chemistry, bioactivity, extraction and analysis of azadirachtin : State-of-the-art. Fitoterapia 134: 141-150 doi: 10.1016/j.fitote.2019.02.006

Forim MR, Costa ES, Da Silva MFDGF, Fernandes JB, Mondego JM and Boiça Junior AL. 2013. Development of a new method to prepare nano-/microparticles loaded with extracts of *Azadirachta indica*, their characterization and use in controlling *Plutella xylostella*. Journal of Agricultural and Food Chemistry. 61(38): 9131–9139. doi. 10.1021/jf403187y

Imam H, Azad H and Ahmed A. 2012. Neem (*Azadirachta indica* A. Juss) - A Nature's Drugstore: An overview. International Research Journal of Biological Sciences 1(6): 76-79.

Krishnaveni S and Ramamurthy V. 2014. Larvicidal efficacy of leaf extracts of *Heliotropium indicum* and *Mukia maderaspatana* against the dengue fever mosquito vector *Aedes aegypti*. Journal of Entomology and Zoology Studies 2(5): 40-45.

Lin T, Liu Q and Chen J. 2016. Identification of differentially expressed genes in *Monochamus alternatus* digested with azadirachtin. Scientific Reports 6: 33484. doi: 10.1038/srep33484

Liu L, Zhao YL, Cheng GG, Chen YY, Qin XJ, Song CW, Yang XW, Liu YP and Luo XD. 2014. Limonoid and steroidal saponin from *Azadirachta indica*. Natural Products and Bioprospecting 4: 335-340. doi: 10.1007/s13659-014-0042-2

Maneemegalai S and Sathish M. 2008. Evaluation of larvicidal effect of *Lantana camara* Linn against mosquito species *Aedes aegypti* and *Culex quinquefasciatus*. Advances in Biological Research 2(3-4): 39–43.

Maragathavalli S, Brindha S, Kaviyarasi NS, Annadurai B and Gangwar SK. 2012. Mosquitoes larvicidal activity of leaf extract of Neem (*Azadirachta Indica*). Advanced Biological Research 2(1): 138-142.

Ministerio de Salud Pública. 2015. Gaceta Epidemiológica Semanal No. 40. Ministerio de Salud Pública, Quito. 48p

Miyazawa M, Anzai J, Fujioka J and Isikawa Y. 2003. Insecticidal compounds against *Drosophila melanogaster* from *Cornus officinalis* Sieb. et Zucc. Natural Product Research 17(5): 337–339. doi: 10.1080/1057563031000072587

Ndione R, Faye O, Ndiaye M, Dieye A and Afoutou J. 2007. Toxic effects of neem products (*Azadirachta indica* A. Juss) on *Aedes aegypti* Linnaeus 1762 larvae. African Journal of Biotechnology 6(24): 2846-2854.

Nour AH, Sandanasamy JDO and Nour AH. 2012. Larvicidal activity of extracts from different parts of Neem (*Azadirachta indica*) against *Aedes aegypti* mosquitoes' larvae. Scientific Research and Essays 7(31): 2810-2815. doi: 10.5897/SRE12.133

PAHO – Pan American Health Organization. 2020. Dengue. In https://www.paho.org/es/temas/dengue Accessed: August 2020.

Pratheeba T, Ragavendran C and Natarajan D 2015. Larvicidal, pupicidal and adulticidal potential of *Ocimum gratissimum* plant leaf extracts against filariasis inducing vector. International Journal of Mosquito Research 2(2): 1–8.

Rahuman AA, Venkatesan P and Gopalakrishnan G, 2008. Mosquito larvicidal activity of oleic and linoleic acids isolated from Citrullus colocynthis (Linn.) Schrad. Parasitology Research 103(6): 1383–1390. doi: 10.1007/s00436-008-1146-6

Renjana PK and Thoppil JE. 2013. Larvicidal activities of the leaf extracts and essential oil of *Premna latifolia* Roxb. (Verbenaceae) against *Aedes albopictus* Skuse (Diptera: Culicidae). Journal of Applied Pharmaceutical Science 3(6): 101–105. doi: 10.7324/JAPS.2013.3616

Schneider LCL, Silva CV and Conte H. 2017. Toxic effect of commercial formulations of neem oil, *Azadirachta indica* A. Juss., in pupae and adults of the sugarcane borer, *Diatraea saccharalis* F. (Lepidoptera: Crambidae). Arquivos do Instituto Biológico 84: e0432014. doi: 10.1590/1808-1657000432014

Shaalan EAS, Canyon D, Younes MWF, Abdel-Wahab H and Mansour AH. 2005. A review of botanical phytochemicals with mosquitocidal potential. Environment International 31(8): 1149-1166. doi: 10.1016/j.envint.2005.03.003

Shamia P, Mohan L and Srivastava CN. 2006. Impact Analysis of Neem Kernel Extracts on the Developmental Profile of *Anopheles stephensi*. Journal of Asia-Pacific Entomology 9(1): 11-17. doi: 10.1016/S1226-8615(08)60270-8

Su T and Mulla MS. 2003. Oviposition bioassay responses of *Culex tarsalis* and *Culex quinquefasciatus* to neem products containing azadirachtin. Entomologia Experimentalis et Applicata 91(2): 337-345. doi: 10.1046/j.1570-7458.1999.00500.x

Umar MI, Asmawi MZ, Sadikun A, Abdul Majid AMS, Atangwho IJ Item M, Khadeer Ahamed MB, Altaf R and Ahmad A. 2014. Multi-constituent synergism is responsible for anti-inflammatory effect of *Azadirachta indica* leaf extract. Pharmaceutical Biology 52(11):1-12. doi: 10.3109/13880209.2014.895017

Vargas-Miranda K, Troyo A y Calderón-Arguedas Ó. 2019. Resistencia de *Aedes aegypti* (Diptera: Culicidae) a insecticidas organofosforados y piretroides en la localidad de Orotina, Alajuela, Costa Rica. Revista Costarricense de Salud Pública 28(1): 15-24.

Vietmeyer ND. 1992. Neem: a tree for solving global problems. Report of an ad hoc panel of the Board on Science and Technology for International Development. National Research Council. National Academy Press, Washington DC.

Wandscheer CB, Duque JE, Da Silva MAN, Fukuyama Y, Wohlke JL, Adelmann J and Fontana JD. 2004. Larvicidal action of ethanolic extracts from fruit endocarps of *Melia azedarach* and *Azadirachta indica* against the dengue mosquito *Aedes aegypti*. Toxicon 44(8): 829-835. doi: 10.1016/j.toxicon.2004.07.009

WHO – World Health Organization. 2009. Dengue Guidelines For Diagnosis, Treatment, Prevention And Control: new edition. WHO Press, Geneva. 147 p.

WHO – World Health Organization. 2020a. Malaria. Insecticide resistance. In. http://www.who.int/malaria/areas/vector_control/insecticide_resistance/en/ Accessed: August, 2020.

WHO – World Health Organization. 2020b. Dengue and severe dengue. In: https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue Accessed: August, 2020.

Zorzetti J, Constanski K, Santoro PH, Fonseca ICB and Neves PMOJ. 2015. Growth regulator insecticides for the control of the lesser mealworm beetle *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). Revista Colombiana de Entomología 41(1): 24–32.

Research article

http://www.revistas.unal.edu.co/index.php/refame

Revista Facultad Nacional de Agronomía

Fruit preservation with bioethanol obtained from the fermentation of brewer's spent grain with *Saccharomyces carlsbergensis*

Preservación de frutas con bioetanol obtenido a partir de la fermentación de cascarilla de cebada cervecera con *Saccharomyces carlsbergensis*

doi: 10.15446/rfnam.v73n3.85316

Clement Olusola Ogidi[1]*, Oluwatobiloba Hannah George[1], Oluwatoyin Modupe Aladejana[2], Olu Malomo[1] and Oladiran Famurewa[2]

Keywords:
Agro-wastes
Bioethanol
Fermentation
Fruit rottenness

ABSTRACT

Brewer's Spent Grain (BSG) is renewable lignocellulosic biomass generated from the beer brewing process. It serves as a substrate for various biotechnological applications. BSG was used as the main substrate for bioethanol production with *Saccharomyces carlsbergensis* in submerged fermentation. Saccharification and fermentation studies were performed for the production of bioethanol. A sterilized fermenter was loaded with 50 g L^{-1} of BSG at 29±2 °C and an agitation speed of 180 rpm. pH was adjusted to 6.0 before the addition of 500 mL of yeast culture for 7 days under submerged and optimized conditions. The fermented product was concentrated using a rotary evaporator at 66±1 °C, and ethanol was qualitatively determined by the dichromate method. Bioethanol yield was 22%, with a specific gravity of 0.8 at 28 °C. Fourier-Transform Infrared Spectroscopy (FTIR) confirmed the presence of $-CH_{3\ stretch}$, $-OH_{stretch}$ and $-CH_{2stretch}$ in bioethanol. For the preservative test, *Staphylococcus* spp., *Erwinia* spp., *Lactobacillus* spp., *Bacillus* spp., *Xanthomonas* spp., *Pseudomonas* spp., *Micrococcus* spp. and *Corynebacterium* spp. were the bacteria isolated from fruits examined from different regions of Osun State. The genera of fungi isolated were *Aspergillus*, *Colletotrichum*, *Penicillium*, *Fusarium*, *Alternaria*, *Rhizopus, Candida*, *Saccharomyces*, *Geotrichium* and *Pichia.* Bioethanol produced from BSG inhibited the growth of microorganisms with zones of inhibition range from 7.0 mm to 11.5 mm, and thus, selected fruits were preserved. Hence, the fermentation technology of agro-industrial wastes with microorganisms can be adopted to convert waste biomass to useful resources.

Palabras clave:
Agro-residuos
Bioetanol
Fermentación
Podredumbre de frutos

RESUMEN

Cascarilla de cebada cervecera (CCC) es una biomasa lignocelulósica renovable generada a partir del proceso de elaboración de la cerveza, que sirve como sustrato para diversas aplicaciones biotecnológicas. Se usó CCC como sustrato principal para la producción de bioetanol con *Saccharomyces carlsbergensis* en fermentación sumergida. Se realizaron estudios de sacarificación y fermentación para la producción de bioetanol, el fermentador esterilizado se cargó con 50 g L^{-1} de CCC a 29±2 °C y una velocidad de agitación de 180 rpm. El pH se ajustó a 6,0 antes de la adición de 500 mL de cultivo de levadura durante 7 días en condiciones sumergidas y optimizadas. El producto fermentado se concentró usando un evaporador rotatorio a 66±1 °C y el etanol se determinó cualitativamente por el método de dicromato. El rendimiento de bioetanol fue del 22% con un peso específico de 0,8 a 28 °C. La Espectroscopía Infrarroja por Transformada de Fourier (FTIR) confirmó la presencia de CH_3, OH y CH_2 en el bioetanol. Para el ensayo de preservación, *Staphylococcus* spp., *Erwinia* spp., *Lactobacillus* spp., *Bacillus* sp., *Xanthomonas* spp., *Pseudomonas* spp., *Micrococcus* spp. y *Corynebacterium* spp. fueron bacterias aisladas de frutas examinadas de diferentes regiones del estado de Osun. Los géneros de hongos aislados fueron *Aspergillus*, *Colletotrichum*, *Penicillium*, *Fusarium*, *Alternaria*, *Rhizopus, Candida*, *Saccharomyces*, *Geotrichium* y *Pichia*. El bioetanol producido a partir de CCC inhibió el crecimiento de microorganismos con zonas de inhibición comprendidas entre 7,0 mm y 11,5 mm conservando las frutas seleccionadas. Por lo tanto, se puede adoptar la tecnología de fermentación de desechos agroindustriales con microorganismos para convertir la biomasa residual en recursos útiles.

[1] Biotechnology Unit, Department of Biological Sciences, Kings University, PMB 555, Odeomu, Nigeria.
[2] Microbiology Unit, Department of Biological Sciences, Kings University, PMB 555, Odeomu, Nigeria.
* Corresponding author: <clementogidi@yahoo.com>

Received: March 30, 2020; Accepted: August 3, 2020
Rev. Fac. Nac. Agron. Medellín 73(3): 9321-9331. 2020
ISSN 0304-2847 / e-ISSN 2248-7026

Wheat bran, rice bran, corn cob and wheat straw are examples of agricultural wastes available as carbon sources (Singh *et al*., 2012). Brewer's Spent Grain (BSG), a waste-product of the mashing process, is one of the initial operations in brewery industries to solubilize malt and grains to ensure adequate extraction of wort (Aliyu and Bala, 2011). BSG is available in larger quantities; approximately 85-90% of the total by-products generated during beer production; however, its central exploitation or disposition has been inadequate (Steiner *et al*., 2015). Several attempts have been made to use BSG in biotechnological processes, and this is achievable in various researches and industries by adopting solid-state fermentation (SFF) or submerged fermentation (SMF) since BSG contains basic nutrients required for microbial growth (Mussatto, 2014). BSG is a lignocellulosic material with 17% cellulose, 28% non-cellulosic polysaccharides, minerals, vitamins, proteins, amino acids, arabinoxylans, 28% lignin (Ivanova *et al*., 2017). Therefore, it has a perspective to be recycled and to become useful products. BSG has been utilized as a new and economical medium for the cultivation of microorganisms (Tan *et al*., 2020).

Agricultural residues are currently utilized for the production of bioethanol to decrease total dependence on forest woody biomass and continuous deforestation. Bioethanol is produced from different agro-wastes using some biotechnological methods such as fermentation with diverse microorganisms (Bušić *et al*., 2018). The use of carbon sources from renewable biomass is economical for the full exploitation of less expensive sources into the production of beneficial products (Saini *et al*., 2015). Hence, agro-industrial residues are an attractive alternative to costly raw materials. Bioethanol has been produced by converting sugars directly from BSG or indirectly through starch into alcohol via fermentation followed by distillation (Azhar *et al*., 2017). Ethanol produced from lignocellulosic biomass raises a global interest because it represents an excellent alternative to petroleum-derived energies and reduces food versus fuel conflict generated by first-generation ethanol (Awoyale and Lokhat, 2019; Prasad *et al*., 2019).

Ethanol is an excellent preservative agent that protects several surface fruits (external morphology) from microbial colonization (Dao and Dantigny, 2011). Hitherto, preservation of fresh fruits and vegetables is among the challenges of food products for commercial producers and distributors, particularly in middle-income, low or poor resource countries. Although many fresh fruits are in ideal conditions to hinder microorganisms from colonizing their integument, a lot of challenges in terms of post-harvest, storage, preservation of fruits or vegetables are rising up. It has generated the search of natural preservatives from plants or agro-wastes as an alternative and safer choice since they displayed little or no side effects (Sagar *et al*., 2018; Saeed *et al*., 2019). The bioethanol produced from agro-waste using *S. carlsbergensis* in submerged fermentation can be used as a preservative agent, which will not only help to reduce wastes in the environment but will preserve fruits or crops from post-harvest spoilage. Hence, this study aimed to produce bioethanol using BSG as a substrate with *S. carlsbergensis* in submerged fermentation and to assess the preservative potential of bioethanol on some selected fruits.

MATERIALS AND METHODS

Collection of brewer's spent grain and brewer's spent yeast

The brewer's spent grain and brewer's spent yeast were obtained from International Breweries Plc., Ilesha in Osun state, Nigeria. The town is located at longitude 7.6395°N and latitude 4.7588°E.

Collection of fruits from various locations in Osun State

Various types of fruits were collected from different locations in Osun State. This State was grouped into four (4) zones, A: Odeomu/Gbongan axis, B: Ife and its environment, C: Osogbo, and D: Ilesha and its environment. A total of 161 different fruits were collected as pineapple (*Ananas comosus*), orange (*Citrus sinensis*), African star apple (*Chrysophyllum albidum*), tomato (*Solanum lycopersicum*), banana (*Musa acuminata*), lime (*Citrus aurantiifolia*), pawpaw (*Carica papaya*), sour-sop (*Annona muricata*), watermelon (*Citrullus lanatus*), apple (*Malus domestica*), plantain (*Musa paradisiaca*) and almond (*Prunus dulcis*).

Source of *S. carlsbergensis*

S. carlsbergensis was isolated from brewer's spent yeast

using serial dilution method. A loop full from 10^{-6} was cultured on Potato Dextrose Agar (PDA) and incubated at 25 °C for 48 h. The yeast was subcultured into another freshly prepared PDA and incubated at 25 °C for 48 h to get pure isolate of *S. carlsbergensis.* The pure isolate was transferred to yeast broth and incubated for 48 h.

Production of bioethanol from BSG

The method, according to Alam *et al*. (2009), was adopted for the production of bioethanol with a slight modification. The fermenter was sterilized using 3% v/v of hypochlorite. The sterile fermenter was loaded with 50 g L^{-1} of BSG at 29±2 °C and an agitation speed of 180 rpm. The pH was adjusted to 6.0 before the addition of 500 mL of yeast culture; the fermentation lasted 7 days under submerged and optimized conditions. The aeration and pH were kept stable during fermentation. The fermented product was concentrated using a rotatory evaporator at 66±1 °C, and ethanol was qualitatively determined by the dichromate method.

FTIR spectroscopic of bioethanol from BSG

Structural analysis of the functional group in bioethanol was determined using FT-IR spectroscopy (8400S, Shimadzu Scientific Instruments Inc.). Briefly, bioethanol (1.0 µL) was placed on a fused KBr disc. This was placed on the cell holder, clamped loosely and fixed on the infrared (IR) beam. The running was done at 400 to 4000 per cm wavenumber.

Isolation and identification of microorganisms from fruits

The surface of fruit was sterilized with 1% v/v hypochlorite and rinsed with sterile distilled water to remove normal flora of fruit and other possible microbial contaminants. Fruit samples were observed for 4-5 days to check any sign of spoilage. The rotten part of fruit was aseptically cut and transferred into sterilized peptone water. The sample was shaken vigorously and then allowed to stand for 30 min (Ajayi-Moses *et al*., 2019). Serial dilution was carried out up to10^{-4} and 10^{-5} dilution factor. An aliquot of 0.1 mL was aseptically transferred into Petri dish, and molten nutrient agar or PDA was then introduced. The plate solidified at room temperature (29±1 °C), then plates were incubated at 37 °C for 24 h and 2-3 days at 25 °C for bacteria and fungi, respectively. Discrete colonies were counted and recorded as colony-forming unit per gram (CFU g^{-1}) for bacteria and spore-forming unit per gram (SFU g^{-1}) for fungi. A pure colony was obtained by subcultured, and isolates were tentatively grouped according to their morphological, cultural, and staining characteristics. Biochemical tests such as the catalase test, production of hydrogen sulfide (H_2S), indole, urease, methyl red, oxidase, coagulase, motility, methyl red, Voges-Proskauer, starch hydrolysis and sugars fermentation were carried out using the methods described by Olutiola *et al*. (2000). The results of the biochemical test were compared to Bergey's Manual of Systematic Bacteriology (Krieg *et al*., 2010). Fungi isolates were identified using cultural and microscopic observations, according to Barnett *et al*. (2000) and Samson *et al*. (2010).

***In vitro* antimicrobial activity of bioethanol and other preservatives against microorganisms**

The antimicrobial activity of the bioethanol against spoilage microorganisms isolated from fruits was performed using agar well diffusion (CLSI, 2014). Suspension of test microorganisms was adjusted by the spectrophotometer to 0.5 McFarland standard. Sterile cotton swabs were dipped in the microbial suspension and spread on the surface of the agar plate. A sterilized cork borer was used for cutting wells in each plate. Bioethanol (50 µL) was introduced and sorbic acid (5.0 mg mL^{-1}), ampiclox (5.0 mg mL^{-1}) and terbinafine (5.0 mg mL^{-1}) were implemented as positive controls against bacteria and fungi, while sterile distilled water was used as the negative control. All the plates were labeled appropriately and incubated at 37 °C during 24 h for bacteria and at 25 °C for 48 h for fungi. The diameter of zones of inhibition around wells was measured in millimeter (mm).

Preservation of fruits using bioethanol from BSG

Most prevalent as well as colonizing microorganisms (1.0×10^5 CFU mL^{-1} or SFU mL^{-1}) were re-introduced to apparently healthy (absence of diseases, no wound symptoms or lesion) selected fruits (orange, watermelon, pineapple, and tomato). Each fruit was discretely remained in a sterile laminar hood without touching each other. After 24 h, bioethanol (20 mL) was applied on the surface fruits of the group A. The bioethanol was adjusted to 40% v/v since Kalathenos and Russell (2003) revealed that <30% v/v were rarely

biocidal. Ethanol ≥70% v/v could cause damage to the fruit integument. Group B was treated with sorbic acid (5% w/v), group C was un-inoculated fruits with microorganism and group D was fruits inoculated with microorganisms but untreated with either bioethanol or sorbic acid. The fruit samples were observed for 7 days at room temperature (29 °C).

Statistical analysis

Data were presented as mean±standard deviation from three repetitions. Data obtained in this study were subjected to One-way Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 20 (USA). For bacteria and fungi count, the mean values were compared by Duncan's new multiple range test (MRT). Differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Yield and physicochemical parameters of bioethanol from BSG with *S. cerevisiae*

Table 1 shows the physicochemical parameters of bioethanol produced from BSG. The yield obtained was 22%, with a specific gravity of 0.8. These results contrast with the findings of Irfan *et al*. (2014). They reported bioethanol from sugarcane bagasse, rice straw and wheat straw by *S. cerevisiae* with a value of 77 g L^{-1}, 62 g L^{-1}, and 44 g L^{-1}, respectively. Ingale *et al*. (2014) obtained ethanol of 17.1 g L^{-1} with a higher yield of 84% from banana pseudostem fermented by *S. cerevisiae* NCIM 3570. The higher proportion of ethanol in their study could be associated with two fungal strains of *Aspergillus* spp., which facilitated the maximal release of sugars to produce more ethanol. The combination of *Aspergillus oryzae* and *S. cerevisiae* NCYC479 produced the highest concentrations of ethanol (37 g L^{-1}) in 10 days from BSG using consolidated bioprocessing (Wilkinson *et al*., 2017). Moodley and Gueguim Kana (2019) optimized pretreatment techniques to produce 25% more bioethanol from sugarcane leaf waste using *S. cerevisiae* BY4743. *S. cerevisiae* is one of the best yeast widely employed for commercial production of bioethanol. *S. cerevisiae* has been attractive for efficient consolidated bioprocessing and several biotechnological purposes because of its novel amylolytic enzyme combination, relatively high tolerance to osmotic stress and anaerobic conditions. Therefore, it is suitable for large-scale fermentation of agro-wastes into bioethanol (Cripwell *et al*., 2019). In the study of Wu *et al*. (2020), replacement of ethanol fermentation-associated regulatory gene in *S. cerevisiae* was reported to enhance ethanol production by a 5.30% increase in yield and 12.5% decrease in fermentation time when compared to the original strain. Another method that could increase the yield of ethanol is mixed substrates. Bolade *et al*. (2019) claimed that multi-substrates biomass of agro wastes increases the yield of bioethanol.

Table 1. Physicochemical properties of bioethanol produced from BSG.

Test	Obtained bioethanol	Ethanol 70% v/v (standard)
Yield (%)	22.0±0.01	-
Specific gravity	0.80±0.00	0.79±0.01
Moisture (%)	6.80±0.00	0.50±0.00
Flammability	Weak	High

-: yield was not quantified; it was used as control.

BSG is interesting biomass with hydrolyzable fermentable sugars that can be converted to ethanol with different microorganisms through co-fermentation strategies (Rojas-Chamorro *et al*., 2020). Likewise, cassava peels, potato peels and millet husks with different microbial inoculants such as *S. cerevisiae*, *Rhizopus nigricans*, *Aspergillus niger*, *Spirogyra africana* showed great potential for bioethanol production (Chibuzor *et al*., 2016). The large proportion of lignocellulosic materials such as corncob, cornstalk, cornhusk, sugarcane bagasse and sugarcane bark that are creating environmental pollution, can be easily degraded by microorganisms and thus, serve as a substrate for renewable resources (bioethanol).

Figure 1 shows the transmittance and peak representing functional groups in the bioethanol. Table 2 shows

various functional groups found in bioethanol, hydroxyl ($OH_{stretch}$), methyl ($CH_{3\ stretch}$), and alkane ($CH_{2\ stretch}$). These functional groups in ethanol (alcohol) give it the biocide property, and it is responsible for the antisepsis, disinfection and can be used as a preservative agent (McDonnell and Russell, 1999). Hydroxyl group in ethanol (alcohol) acts as an antimicrobial agent against microbes except for alcohol-tolerant strains. It causes a partial breakdown of membrane function, inhibiting cell growth or protein synthesis, denaturation of proteins, and membrane damage, which lead to cell perturbations like ion leakage or loss of energy (Horinouchi *et al*., 2018).

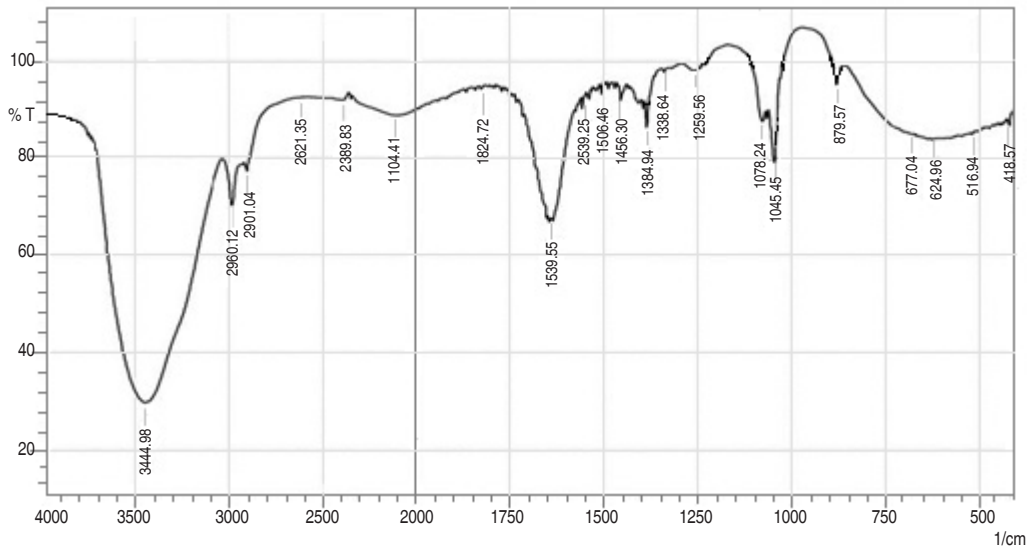


Figure 1. FTIR spectrum of bioethanol produced from BSG

Table 2. The functional group identified in bioethanol produced from BSG.

Sample	Wavenumber (cm^{-1})	Functional group	Name of group
Bioethanol	3444.98	$-OH_{stretch}$	Hydroxyl
	2621.36	$-CH_{3\ stretch}$	Methyl
	1539.45	$-CH_{2\ stretch}$	Alkane

Total microbial load and occurrence (%) of microorganisms isolated from selected fruits

The colonies counted from each fruit are shown in Table 3. The highest colonies of bacteria count (1.05×10^5 CFU g^{-1}) were recorded for bananas from Odeomu (A) market ($P<0.05$). Pineapple from Ilesha had the highest fungal count (1.96×10^6 SFU g^{-1}). Watermelon from Odeomu (A) had the least bacterial load ($P<0.05$) of 1.0×10^4 CFU g^{-1}, and African star apple collected from Osogbo (C) had the least fungal count of 1.1×10^5 SFU g^{-1}. Ajayi-Moses *et al*. (2019) reported the highest bacterial count of 5.84×10^5 CFU g^{-1} for tomatoes and African star apple with the highest fungal count of 3.04×10^5 SFU g^{-1}. Evaluation of microorganisms associated with fruits revealed that various bacteria and fungi in high densities could spoil fruits. Spoilage microorganisms can also colonize, enter and penetrate plant tissues at fruit development, either through calyx, stem or various specialized water and gas exchange structures of leafy matter. However, successful establishment requires the spoilage microbe to overcome multiple natural protective barriers and other factors (Erkmen and Bozoglu, 2016). Lime and soursop did not show any sign of spoilage, and no microorganisms were isolated (Table 3). Fruits like lime and soursop with higher pKa do not get spoilt but rather get dehydrated, exiting fruit juice, a process attributed to spoilage organisms affecting fruits (Czajkowski *et al*., 2011).

Table 3. Bacteria and fungi count from fruits obtained from different regions in Osun State.

Fruits	Locations							
	A	B	C	D	A	B	C	D
	Bacteria $\times 10^4$ CFU g^{-1}				Fungi $\times 10^5$ SFU g^{-1}			
Pineapple	5.2±0.0 c	NC	8.5±0.0 d	3.3±0.0 b	7.5±0.0 b	NC	9.0±1.0 d	19.6±2.1 c
Orange	8.4±0.0 d	6.4±0.3 c	5.3±0.1 c	-	7.0±0.1 b	6.6±0.3 a	7.0±0.4 c	NC
African star apple	8.4±0.2 d	NC	5.6±0.0 c	NC	7.5±0.2 b	0.0	1.1±0.0 a	-
Tomato	7.2±0.4 d	4.7±0.1 a	5.2±0.0 c	1.1±0.0 a	5.5±0.4 a	7.0±0.1 a	8.0±0.3 c	6.5±0.0 b
Banana	10.5±1.0 e	0.0	4.2±0.0 b	1.3±0.0 a	8.0±1.0 c	NC	7.5±0.0 c	2.2±0.1 a
Lime	-	-	-	-	-	-	-	-
Pawpaw	3.0±0.0 b	7.7±0.2 c	1.0±0.0 a	1.0±0.0 a	9.0±0.0 c	6.5±0.0 a	5.0±0.0 c	6.2±0.0 b
Sour sop	-	-	-	-	-	-	-	-
Watermelon	1.0±0.0 a	5.2±0.2 b	4.9±0.0 b	NC	-	7.0±0.0 b	8.0±0.0 c	NC
Almond	NC	5.3±0.0 b	NC	NC	NC	8.0±0.0 b	NC	NC
Apple	-	-	-	-	-	-	-	-
Plantain	NC	NC	-	1.4±0.0 a	NC	NC	-	1.7±0.0 a

Values with different letters along the same column are significantly different (P<0.05).
A: Odeomu/Gbongon axis, B: Ife, C: Osogbo and D: Ilesha.
-: No microbial growth; NC: Fruit samples were not collected.

Table 4 shows the percentage of the occurrence of bacteria. The highest percentage of occurrence (25.6%) was obtained for *Corynebacterium* sp. followed by *Lactobacillus* sp. with 17.9%. The lowest bacteria percentage occurrence (5.1%) was obtained for species of *Micrococcus* and *Staphylococus*. *Erwinia* spp. were found to be associated with spoilage of pineapple, African star apple, tomato, watermelon and almond. The bacterium *Erwinia carotovora* subsp. carotovora is a highly effective spoilage microorganism that produces an increasing amount of pectolytic enzymes to degrade fruit tissues. It causes soft rot on fruits like oranges, tomatoes, banana, pineapple, and watermelon (Barth et al., 2009; Sharma et al., 2013). Besides, *E. carotovora*, several *Pseudomonas* spp., *Corynebacterium*, *Xanthomonas campestris* and lactic acid bacteria are important spoilage bacteria of fruits (Tournas, 2005; Erkmen and Bozoglu, 2016). Some spoilage microbes are capable of colonizing, creating lesions and damaged healthy plant tissues. The type of microbial spoilage in fruits is based on the pH, nutrient availability, water activity (a_w), temperature, relative humidity, oxidation-reduction potential and content of biological structure of fruits (Erkmen and Bozoglu, 2016).

Table 4. Percentage of occurrence of bacteria isolated from different locations

Bacterial isolates	Pa	O	Asa	T	B	L	P	Wm	Ss	Al	Pl	Ap	N	%
Corynebacterium sp.	+	+	+	+	+	-	+	+	-	+	+	-	10	25.6
Lactobacillus sp.	+	+	+	+	+	-	-	-	-	+	+	-	7	17.9
Bacillus sp.	-	-	+	-	+	-	+	-	-	+	+	-	5	12.8
Xanthomonas sp.	+	-	-	+	+	-	-	+	-	+	-	-	5	12.8
Erwinia sp.	+	-	+	+	-	-	-	+	-	+	-	-	5	12.8
Pseudomonas sp.	+	-	-	+	+	-	-	-	-	-	-	-	3	7.7
Micrococcus sp.	+	-	+	-	-	-	-	-	-	-	-	-	2	5.1
*Staphylococ*us sp.	+	-	-	-	-	-	-	-	-	-	+	-	2	5.1

Pa: Pineapple; O: Orange; Asa: African star apple; T: Tomato; B: Banana; L: Lime; P: Pawpaw; Wm: Watermelon; Ss: Sour sop; Al: Almond; Pl: Plantain; Ap: Apple; N: number of isolates.
+: presence of bacteria; -: absence of bacteria.

Fungi with the highest percentage of occurrence was *Aspergillus niger* (13.9%), followed by *Colletotrichum* sp. and *Penicillium digitatium* with the same value of 11.1%. *Pichia* sp. has the lowest percentage of occurrence of 4.2% (Table 5). The highest occurrence of *A. niger* from examined fruits in Osun State is in concordances with findings of Mailafia *et al.* (2017). Researchers revealed that *Aspergillus spp.* had the highest occurrence in fruits like pineapple, watermelon, oranges, pawpaw, and tomatoes with a frequency of 38%, followed by *Fusarium avenaceum* with occurrence of 31% in pineapple, watermelon, oranges, pawpaw and tomatoes. In comparison, *P. digitatum* and *R. stolonifera* have the least frequency at the same value of 4% for tomato and orange. Other fungal species identified as agents of spoilage were *Saccharomyces* spp. (10%), *F. solani (*8%), and *A. flavus* (5%). Some of the fungi isolated from fruits were species of *Penicillum*, *Aspergillus*, *Rhizopus*, *Fusarium* and *Mucor iriformis*. Tafinta *et al.* (2013) and Ajayi-Moses *et al.* (2019) isolated similar microorganisms with varying prevalence from banana, pawpaw, orange, tomato, apple, pineapple, watermelon, cucumber, and African star apple. Some of these fungal isolates are known to be pathogenic due to the toxic secondary metabolites produced. *Penicillium expansum* and *Botrytis cinerea* are pathogenic spoilage microorganisms, which cause blue-rot, grey mold in African star apple, cherry, apple, tomato, pears and kiwi fruit called sour sop (Miedes and Lorences, 2004).

Table 5. Percentage of occurrence of fungi isolated from different locations

Fungi isolates	Pa	O	Asa	T	B	L	P	Wm	Ss	Al	Pl	Ap	N	%
Aspergillus niger	+	+	+	+	+	-	+	-	+	+	+	-	10	13.9
Colletotrichum sp.	+	-	+	+	+	-	-	-	+	+	+	-	8	11.1
Penicillium digitatium	-	+	+	+	+	-	+	-	-	-	+	-	8	11.1
Saccharomyces sp.	+	+	+	+	-	-	-	-	-	-	-	-	6	8.3
Penicillium italicium	+	-	-	-	+	-	+	-	-	+	+	-	6	8.3
Candida tropicalis	+	+	+	+	+	-	-	-	-	+	-	+	6	8.3
Geotrichium sp.	+	+	-	+	+	-	+	-	-	-	+	-	6	8.3
Fusarium sp.	+	+	-	+	+	-	-	-	-	-	-	-	5	6.9
Alternaria sp.	+	-	-	+	+	-	-	-	-	-	-	-	4	5.6
Rhizopus sp.	-	-	-	+	+	-	+	-	-	-	+	-	4	5.6
Pichia sp.	-	-	-	-	-	-	+	-	-	-	-	-	3	4.2

Pa: Pineapple; O: Orange; Asa: African star apple; T: Tomato; B: Banana; L: Lime; P: Pawpaw; Wm: Watermelon; Ss: Soursop; Al: Almond; Pl: Plantain; Ap: Apple; N: number of isolates.
+: presence of fungi; -: abscence were absent

Likewise, molds of genera *Rhizopus*, *Alternaria* and *Botrytis* produce acidic compounds that cause fruit and vegetable rot with distorted color, texture and or taste (Tournas, 2005). Fernández-Cruz *et al.* (2010), Lewis and Goodrich-Schneider (2012) revealed different species of fungi that produce mycotoxins (aflatoxins, ochratoxin, patulin, fumonisin, alternariol, alternariol methyl ether, and altenuene) in fruits. The problem associated with mycotoxins in fruits includes economic loss, poor organoleptic properties, toxicities (acute to chronic), and a spectrum of effect (mild to severe), including carcinogenicity and death. Spoilage microorganisms exploit fruit components using their extracellular lytic enzymes, pectinases and hemicellulases to degrade fruit polymers to release intracellular constituents as nutrients for growth (Kalia and Gupta, 2006). Microorganisms from fruits secreted a wide variety of enzymes. Isolation and identification of novel strains of microorganism from fruits can serve as natural origin, a safer and cheaper alternative source of microbial enzymes as promising candidates for biotechnological uses and medical processes (Sharma *et al.*, 2013; Garg *et al.*, 2016).

Antimicrobial and preservative properties of bioethanol from BSG

To minimize wastage recorded on fruits and vegetables and to reduce economic losses associated with microbial spoilage, a reliable and supportive measure

needs to be sourced. Bioethanol from BSG displayed zones of inhibition against bacteria with values ranging from 7.0 to 8.8 mm, sorbic acid was within 6.0 to 13.0 mm, and ampiclox was from 8.0 to 18.5 mm (Table 6). Zones of inhibition by bioethanol against fungi ranged from 7.5 to 11.5 mm, while sorbic acid was 7.5 to 13.0 mm and 10.0 to 17.0 mm for terbinafine (Table 6). Ethanol is used as a disinfectant and acts against microorganisms in two different ways: growth inhibition (bacteriostasis, fungistasis) or lethal action (bactericidal, fungicidal or virucidal effects) (Maris, 1995). Hence, bioethanol can be used to prevent the growth of spoilage microorganisms on fruits. Ethanol interacts with cell surface followed by penetration into cells and acts on the target site(s) of microorganisms (Maris, 1995). Inactivation of fungal spores, suppression of fungal growth or their germination on fruits by ethanol reflects the inhibitory potential of bioethanol in controlling fruit decaying by fungi and extending the shelf-life of food products (Dao and Dantigny, 2011).

Table 6. Zones of inhibition (mm) displayed by bioethanol and selective preservatives against #microorganisms isolated from fruits.

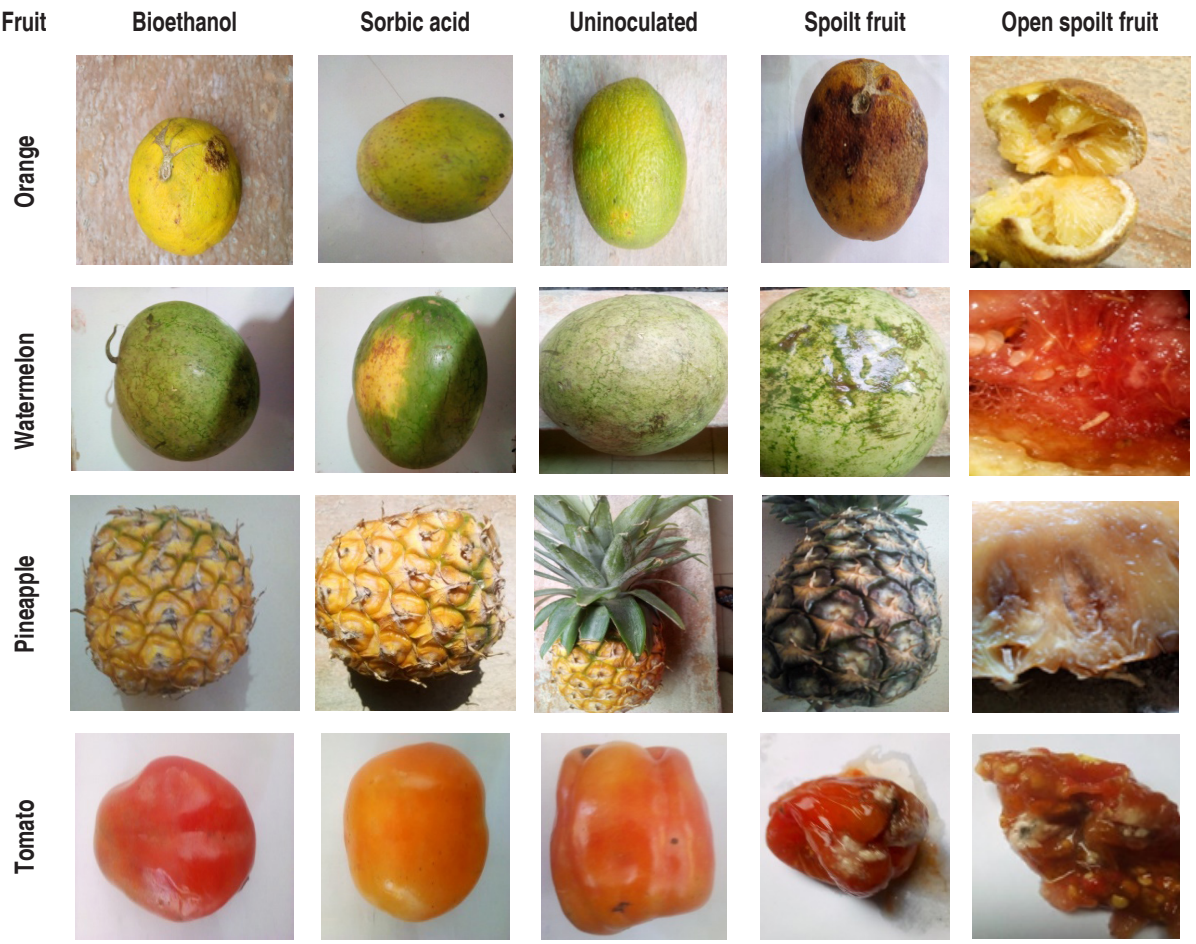
Bacteria isolates	Bioethanol	Sorbic acid	Ampiclox
Corynebacterium sp.	8.0±0.0	10.0±0.2	17.1±2.2
Lactobacillus sp.	8.0±0.1	12.0±0.5	14.5±1.7
Bacillus sp.	8.8±0.0	11.0±0.0	18.0±1.0
Xanthomonas sp.	-	13.0±0.3	16.7±2.1
Erwinia spp.	-	12.0±0.8	18.5±0.8
Pseudomonas sp.	7.0±0.0	-	17.2±1.1
Micrococcus sp.	-	-	8.0±0.0
*Staphylococu*s sp.	-	6.0±0.0	8.0±0.0
Fungal isolates	**Bioethanol**	**Sorbic acid**	**Terbinafine**
Aspergillus niger	10.5±1.0	11.0±1.0	16.2±3.0
Colletotrichum sp.	7.5±0.0	13.0±1.7	17.0±2.3
Penicillium italicium	10.5±0.4	7.5±0.1	11.0±1.0
Saccharomyces sp.	11.5±0.8	8.6±0.3	16.0±1.0
Penicillium digitatum	8.0±0.0	10.3±1.2	10.0±0.0

-: There were no zones of inhibition

Microorganisms able to colonize fruits with spoilage attributes were used as indicator microorganisms.

Table 7 shows different fruits preserved with bioethanol, sorbic acid, un-inoculated fruit with the microorganism, spoilt fruit and open spoilt fruit after 7 days. Dao and Dantigny (2011), found that oranges were protected from fungal infection for 30 days when exposed to 20-100% ethanol. The use of bioethanol as a preservative is important to prevent a higher loss of fruits since it has no side effects on humans. It will proffer the solution to longstanding spoilage of vegetables and fruits, which had been associated with different microorganisms. Ethanol exerts its most effective bactericidal action at $\geq$40 to 95% v/v against vegetative cells like chemical disinfectants (Kalathenos and Russell, 2003). Findings of Katsinis *et al*. (2008) established effective preservation with the synergistic effect of conventional chemical preservative (potassium sorbate) and ethanol, which improved the shelf life of bread by suppressing microbial growth (43.5% and 38.5% mold-free shelf life). Bioethanol displayed a higher zone of inhibition (11.5 mm) against *Saccharomyces* sp. than sorbic acid (8.6 mm). Sorbic acids had less or no inhibitory activity against *S. cerevisiae*, *Saccharomycodes ludwigii*, *Zygosaccharomyces bailii*, *Zygosaccharomyces rouxii*, *Schizosaccharomyces pombe Brettanomyces* spp., *Pichia membranifaciens*, *Dekkerae* spp, and *Issatchenkia orientalis* at 800 mg kg^{-1}, which is much higher than the permissible limit in foods, but ethanol (>20% v/v) exerts a strong lethal effect on these molds (Loureiro and Malfeito-Ferreira, 2003).

Table 7. Evolution of fruits during 7-day treatment with bioethanol and sorbic acid.



The mechanisms of resistance by yeasts to weak organic acids are by inducing the expression of H^+ ATPases to regulate their cytosolic pH, using their plasma membrane components to modulate the influx of lipophilic weak organic acids (Ullah *et al*., 2012). ATP binding cassette (ABC) transporter (Pdr12) prevents anion accumulation or degrade sorbic acid to 1,3-pentadiene (Casas *et al*., 2004). Findings of Linares-Morales *et al.* (2018) revealed that chemical preservatives like organic acids in fruits and vegetables cause disruption of membrane permeability, reduce cell's internal pH, affects metabolic enzymes as well as protein synthesis. However, a microbial product like bioethanol can be encouraged in use as preservative agent since it strongly suppresses microbial activity on fruits. Microbial by-products are bio-protective or natural preservatives, and thus, exhibit antifungal activities. There is a growing interest in alternatives to preservation other than chemical or synthetic agents with different side effects (Leyva Salas *et al*., 2017). The microbial growth inhibition or killing action of bioethanol on microorganisms is an indication that; bioethanol produced by *S. cerevisiae* from renewable biomass (lignocellulosic wastes) is needed for commercial purposes.

CONCLUSION

The bioethanol produced from the fermentation of brewer's spent grain with *S. carlsbergensis* inhibited microorganisms associated with post-harvest spoilage

of fruits. The presence of functional groups in bioethanol contributed to its bioactivity, which makes it a potential preservative agent to suppress the colonization of microbial spoilage on fruits, reaching inhibitions of 8.8 and 11.5 mm for bacteria and fungi, respectively. This study proffer solution to the underutilized BSG residue, which can be used for bioethanol and serve as a preservative agent for fruits or vegetables. However, modern facilities to increase the yield of bioethanol from agro wastes need to be considered in subsequent works.

REFERENCES

Ajayi-Moses OB, Ogidi CO and Akinyele BJ. 2019. Bioactivity of *Citrus* essential oils (CEOs) against microorganisms associated with spoilage of some fruits. Chemical and Biological in Technologies Agricultures 6(22): 15. doi: 10.1186/s40538-019-0160-5

Alam MZ, Kabbashi NA and Hussi SNIS. 2009. Production of bioethanol by direct bioconversion of oil-palm industrial effluent in a stirred-tank bioreactor. Journal of Industrial Microbiology and Biotechnology 36(6): 801-808. doi: 10.1007/s10295-009-0554-7

Aliyu S and Bala M. 2011. Brewer's spent grain: a review of its potentials and applications. African Journal of Biotechnology 10(3): 324-331. doi: 10.5897/AJB10.006

Azhar SHM, Abdulla R, Jambo SA, Marbawi H, Gansau JA, Faik AAM and Rodrigues KF. 2017. Yeasts in sustainable bioethanol production: a review. Biochemistry and Biophysics Reports 10: 52–61. doi: 10.1016/j.bbrep.2017.03.003

Awoyale AA and Lokhat D. 2019. Harnessing the potential of bio-ethanol production from lignocellulosic biomass in Nigeria – a review. Biofuels, Bioproducts and Biorefining 13(1): 192-207. doi: 10.1002/bbb.1943

Barnett JA, Payne RW and Yarrow O. 2000. Yeast: Characteristics and identification. Third Edition. Cambridge University Press, London. pp 12-87.

Barth M, Hankinson TR, Zhuang H and Breidt F. 2009. Microbiological spoilage of fruits and vegetables pp. 135–183. In: Sperber WH and Doyle MP (eds.). Compendium of the microbiological spoilage of foods and beverages: Food Microbiology and Food Safety. Springer, New York. doi: 10.1007/978-1-4419-0826-1_6

Bušić A, Marđetko N, Kundas S, Morzak G, Belskaya H, Ivančić Šantek M, Komes D, Novak S and Šantek B. 2018. Bioethanol production from renewable raw materials and its separation and purification: a review. Food Technology and Biotechnology 56(3): 289–311. doi: 10.17113/ftb.56.03.18.5546

Casas E, de Ancos B, Valderrama MJ, Cano P and Peinado JM. 2004. Pentadiene production from potassium sorbate by osmotolerant yeasts. International Journal of Food Microbiology 94(1): 93–96. doi: 10.1016/j.ijfoodmicro.2004.01.001

Chibuzor O, Uyoh EA and Igile G. 2016. Bioethanol production from cassava peels using different microbial inoculants. African Journal of Biotechnology 15(30): 1608-1612. doi: 10.5897/AJB2016.15391

CLSI - Clinical and Laboratory Standards Institute. 2014. Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement. M100 – S24. Wayne, USA. pp. 1-16.

Czajkowski MC, Perombelon JA, Veen JM and Van der W. 2011. Control of blackleg and tuber spoilage of potatoes caused by *Pectobacterium* and *Dickeya* species: a review. Plant Pathology 60(6): 999-1013. doi: 10.1111/j.1365-3059.2011.02470.x

Dao T and Dantigny P. 2011. Control of food spoilage fungi by ethanol. Food Control 22(3-4): 360-368. doi: 10.1016/j.foodcont.2010.09.019

Erkmen O and Bozoglu TF. 2016. Chapter 20: Spoilage of vegetables and fruits. pp 337-363. In: Food microbiology: principles into practice. doi: 10.1002/9781119237860.ch20

Fernández-Cruz ML, Mansilla ML and Tadeo JL. 2010. Mycotoxins in fruits and their processed products: analysis, occurrence and health implications. Journal of Advanced Research 1(2): 113-122. doi: 10.1016/j.jare.2010.03.002

Garg G, Singh A, Kaur A, Singh R, Kaur J and Mahajan R. 2016. Microbial pectinases: an ecofriendly tool of nature for industries. 3 Biotech 6: 47. doi: 10.1007/s13205-016-0371-4

Horinouchi T, Maeda T and Furusawa C. 2018. Understanding and engineering alcohol-tolerant bacteria using OMICS technology. World Journal of Microbiology and Biotechnology 34: 157. doi: 10.1007/s11274-018-2542-4

Ingale S, Joshi SJ and Gupte A. 2014. Production of bioethanol using agricultural waste: banana pseudo stem. Brazilian Journal of Microbiology 45(3): 885-892. doi: 10.1590/S1517-83822014000300018

Irfan M, Nadeem M and Syed Q. 2014. Ethanol production from agricultural wastes using *Saccharomyces cerevisiae*. Brazilian Journal of Microbiology 45(2): 457–465. doi: 10.1590/s1517-83822014000200012

Ivanova K, Denkova R, Kostov G, Petrova T, Bakalov I, Ruscova M and Penov N. 2017. Extrusion of brewers' spent grains and application in the production of functional food. Characteristics of spent grains and optimization of extrusion. Journal of The Institute of Brewing 123(4): 544-552. doi: 10.1002/jib.448

Kalathenos P and Russell NJ. 2003. Ethanol as a food preservative. pp 196-217. In: Russell NJ and Gould GW. (eds.) Food preservatives. Springer, Boston. doi: 10.1007/978-0-387-30042-9_10

Kalia A and Gupta R. 2006. Fruit Microbiology. pp. 1-28. In: Hui YH (ed.). Handbook of fruit and fruit processing. First Edition. Blackwell publishing, United Kingdom. doi: 10.1002/9780470277737.ch1

Katsinis G, Rigas F and Doulia D. 2008. Synergistic effect of chemical preservatives with ethanol on the microbial shelf life of bread by factorial design. International Journal of Food Science and Technology 43(2): 208-215 doi: 10.1111/j.1365-2621.2006.01386.x

Krieg NR, Staley JT, Brown DR, Hedlund BP, Paster BJ, Ward NL, Ludwig W and Whitman WB. 2010. Bergey's Manual of Systematic Bacteriology. Second edition. Springer, New York. pp. 40-91. doi: 10.1007/978-0-387-68572-4

Lewis DC and Goodrich-Schneider R. 2012. Mycotoxins in fruit and fruit products. Proceedings of the Florida State Horticultural Society. 125:252–257.

Leyva Salas M, Mounier J, Valence F, Coton M, Thierry A and Coton E. 2017. Antifungal microbial agents for food biopreservation- A review. Microorganisms 5(3): 37. doi: 10.3390/microorganisms5030037

Linares-Morales JR, Gutiérrez-Méndez N, Rivera-Chavira BE, Pérez-Vega SB and Nevárez-Moorillón GV. 2018. Biocontrol processes in fruits and fresh produce, the use of lactic acid bacteria as a sustainable option. Frontiers in Sustainable Food System 2:50. doi: 10.3389/fsufs.2018.00050

Loureiro V and Malfeito-Ferreira M. 2003. Spoilage yeasts in the

wine industry. International Journal of Food Microbiology 86(1-2): 23-503. doi: 10.1016/S0168-1605(03)00246-0

Mailafia S, Okoh GR, Olabode H and Osanupin R. 2017. Isolation and identification of fungi associated with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria. Veterinary World 10(4): 393–397. doi: 10.14202/vetworld.2017.393-397

Maris P. 1995. Modes of action of disinfectants. Revue scientifique et technique (International Office of Epizootics) 14 (1):47-55. https://doc.oie.int/dyn/portal/index.seam?page=alo&aloId=27748

McDonnell G and Russell AD. 1999. Antiseptics and disinfectants: activity, action, and resistance. Clinical Microbiology Reviews 12(1): 147-179. doi: 10.1128/CMR.12.1.147

Miedes E. and Lorences EP. 2004. Apple (*Malus domestica*) and tomato (*Solanum lycopersicum*) fruits cell-wall hemicelluloses and xyloglucan degradation during *Penicillium expansum* infection. Journal of Agricultural and Food Chemistry. 52(26): 7957–7963. doi: 10.1021/jf048890f

Moodley P and Gueguim Kana EB. 2019. Bioethanol production from sugarcane leaf waste: Effect of various optimized pretreatments and fermentation conditions on process kinetics Biotechnology Reports 22: e00329. doi: 10.1016/j.btre.2019.e00329

Mussatto SI. 2014. Brewer's spent grain: a valuable feedstock for industrial applications. Journal of the Science of Food and Agriculture 94(7): 1264-1275. doi: 10.1002/jsfa.6486

Olutiola PO, Famurewa O and Sonntag HG. 2000. An introduction to General Microbiology, a practical approach. Heldelberg, Nigeria. pp. 1-267.

Prasad RK, Chatterjee S, Mazumder PB, Gupta SK, Sharma S, Vairale MG, Datta S, Dwivedi SK and Gupta DK. 2019. Bioethanol production from waste lignocelluloses: A review on microbial degradation potential. Chemosphere 231: 588-606. doi: 10.1016/j.chemosphere.2019.05.142

Rojas-Chamorro JA, Romero-García JM Cara C, Romero I and Castro. E. 2020. Improved ethanol production from the slurry of pretreated brewers' spent grain through different co-fermentation strategies. Bioresource Technology 296: 122367. doi: 10.1016/j.biortech.2019.122367

Saeed F, Afzaal M, Tufail T and Ahmad A. 2019. Use of natural antimicrobial agents: a safe preservation approach. In: Var I and Uzunhu S (eds.). Active antimicrobial food packaging. IntechOpen, London. doi: 10.5772/intechopen.80869

Sagar NA, Pareek S, Sharma S, Yahia EM and Lobo MG. 2018. Fruit and vegetable waste: bioactive compounds, their extraction, and possible utilization. Comprehensive Reviews in Food Science and Food Safety 17(3): 512-531. doi: 10.1111/1541-4337.12330

Saini JK, Saini R and Tewari L. 2015. Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: concepts and recent developments. 3 Biotech 5: 337–353 doi: 10.1007/s13205-014-0246-5

Samson RA, Houbraken J, Thrane U, Frisvad JC and Andersen B. 2010. Fungi and indoor fungi. CBS-KNAW Fungal Biodiversity Centre, Utrecht. pp.1-390.

Sharma N, Rathore M and Sharma M. 2013. Microbial pectinase: sources, characterization and applications. Review in Environmental Science and Biotechnology 12: 45–60. doi: 10.1007/s11157-012-9276-9

Singh R, Kapoor V and Kumar V. 2012. Utilization of agro-industrial wastes for the simultaneous production of amylase and xylanase by thermophilic Actinomycetes. Brazilian Journal of Microbiology 43(4): 1545–1552.

Steiner J, Procopio S and Becker T. 2015. Brewer's spent grain: source of value-added polysaccharides for the food industry in reference to the health claims. European Food Research and Technology 241(3): 303-315. doi: 10.1007/s00217-015-2461-7

Tafinta IY, Shehu K, Abdulganiyyu H, Rabe AM and Usman A. 2013. Isolation and identification of fungi associated with the spoilage of sweet orange (*Citrus sinensis*) fruit in Sokoto State. Nigerian Journal of Basic and Applied Sciences 21(3): 193-196. doi: 10.4314/njbas.v21i3.4

Tan YX, Mok WK and Chen WN. 2020. Potential novel nutritional beverage using submerged fermentation with *Bacillus subtilis* WX-17 on brewers' spent grains, Heliyon 6(6): e04155. doi: 10.1016/j.heliyon.2020.e04155

Tournas VH. 2005. Spoilage of vegetable crops by bacteria and fungi and related health hazards. Critical Reviews in Microbiology 31(1): 33-44. doi: 10.1080/10408410590886024

Ullah A, Orij R, Brul S and Smits GJ. 2012. Quantitative analysis of the modes of growth inhibition by weak organic acids in *Saccharomyces cerevisiae*. Applied Environmental Microbiology 78: 8377–8387. doi: 10.1128/AEM.02126-12

Wilkinson S, Smart KA, James S and Cook DJ. 2017. Bioethanol production from brewers spent grains using a fungal consolidated bioprocessing (CBP) approach. Bioenergy Research 10(1): 146-157. doi: 10.1007/s12155-016-9782-7

Research article

http://www.revistas.unal.edu.co/index.php/refame

Revista Facultad Nacional de Agronomía

Effect of a total substitution of vegetable protein and phosphates on shrinkage by cooking and purging in chopped york ham

Efecto de la sustitución total de la proteína vegetal y de los fosfatos sobre las mermas por cocción y las purgas en jamones de cerdo picados tipo york

doi: 10.15446/rfnam.v73n3.80131

José Alfonso Cardona-Hincapié[1]*, Diego Alonso Restrepo-Molina[1] and Jairo Humberto López-Vargas[1]

Keywords:
Brine
Clean labeling
Citrus fiber
Meat products
Polyphosphates

ABSTRACT

The trend with the most significant impact on food is currently clean labeling, and meat products are not exempt from it. This trend promotes the elimination of additives of inorganic origin and their replacement by natural ingredients in the formulation of products. In the present work, the effects of the total substitution of polyphosphate and vegetable protein for citric fiber and hydrolyzed pork collagen in chopped pork York ham, with an extension of 52.9% at the end of cooking, were evaluated to achieve clean labeling. Two treatments were performed with two types of brine, which had a citrus fiber A and a citrus fiber B as phosphate replacements. Additionally, as a vegetable protein replacement, the same hydrolyzed pork collagen was used for both treatments. Tumbler massaging was made to allow correcting protein extraction, then it was subjected to heat treatment by immersion in hot water at 80 °C. It was concluded that the ham made with citric fiber B and hydrolyzed pork collagen obtained better results in texture, syneresis, sensory analysis and cooking losses, with no significant differences with the standard.

Palabras clave:
Salmuera
Etiquetado limpio
Fibra cítrica
Productos cárnicos
Polifosfatos

RESUMEN

La tendencia de mayor impacto en alimentos actualmente es la del etiquetado limpio, y los productos cárnicos no están exentos de ella. Esta tendencia lo que promueve es la eliminación de aditivos de origen inorgánico para ser reemplazados por ingredientes naturales en la formulación de los productos. En el presente trabajo, se evaluaron los efectos que tuvo la sustitución total de polifosfatos y proteína vegetal por fibra cítrica y colágeno hidrolizado de cerdo en jamones de cerdo picados tipo york, con una extensión de 52.9% al final de cocción, con el fin de alcanzar la limpieza de la etiqueta. Se hicieron dos tratamientos con dos tipos de salmuera, las cuales tenían como reemplazante de fosfatos, una fibra cítrica A y una fibra cítrica B; adicionalmente como reemplazante de proteína vegetal se utilizó el mismo colágeno hidrolizado de cerdo para ambos tratamientos. Se llevó a cabo masajeo en tombler para permitir correcta extracción de proteína y tratamiento térmico por inmersión en agua caliente a 80 °C. Se concluyó que el jamón al que se le aplicó fibra cítrica B junto con el colágeno hidrolizado de cerdo, obtuvo mejores resultados en textura, sinéresis, análisis sensorial y mermas por cocción, sin presentar diferencias significativas con el control.

[1] Facultad de Ciencias Agrarias. Univesidad Nacional de Colombia. AA. 1779, Medellín, Colombia.
* Corresponding author: <jacardonah@unal.edu.co>

Received: March 16, 2020; Accepted: July 16, 2020.
Rev. Fac. Nac. Agron. Medellín 73(3): 9333-9340. 2020

ISSN 0304-2847 / e-ISSN 2248-7026

Meat products or cold meat inlay have been designated as "not recommended" foods over time because they contain high levels of fat, sodium and additives. Currently, these products are evolving in accordance with global policies on food, health and nutrition, which establish clear guidelines for food production aimed at making them safer for consumer's health (Moreno, 2013).

Globally, processed meat products (PMP) aim at reducing concentrations of sodium, fat, nitrite, phosphates from meat formulations since they can contribute to developing non-communicable diseases (Restrepo and López, 2013), which, according to the World Health Organization, are equivalent to 71% of the deaths in the world (WHO, 2018). The European Food Safety Authority (EFSA) recommends not exceeding the doses required in meat formulations because this can trigger problems related to cancer (ANS, 2010).

Currently, the industry is working on changing this concept with consumer education and product proposals that, from their design, are intended to be healthier; controlling, for example, the amount of fat they contain, finding products with up to 99% fat-free (Moreno, 2013). Several techniques have been developed for the replacement of phosphates, ranging from partial or total elimination, for the application of new technologies such as high pressures to avoid the use of this additive. A big obstacle presented by the use of these technologies is related to meat protein functional properties because phosphates help improve texture, cohesiveness, water holding, and to reduce salt concentrations (Resconi *et al.*, 2015). Other controversial additives, like nitrates, are a multifunctional ingredient in matured and cooked meat products such as minced or York ham; however, it performs its functions at extremely low concentrations (Restrepo, 2018). This additive is responsible for the color, odor, and cured taste (Honikel, 2004) to protect the flavor avoiding rancidity (as an antioxidant) (Sebranek, 2009) and among other functions to inhibit microbial growth of anaerobic bacteria, especially *Clostridium botulinum*. It also contributes to the control of other pathogenic microorganisms such as *Listeria monocytogenes* (Restrepo, 2018).

Regarding the application of starches in meat products, different concentrations and cooking temperatures can be used, allowing the retention of moisture and providing other characteristics that help meat products functionality such as texture, sensory characteristics, color, among others (Resconi e*t al.*, 2016). Therefore, different origin starches are being used to replace phosphates in order to analyze their behavior in the meat matrix and establish if they comply with the parameters to be classified as clean labeling.

The rejection or acceptance of food is directly related to the ingredients specified on the label, so those that are more familiar to consumers tend to be chosen. In the case of vegetable protein, the discrimination in the purchase of products containing it is due to the excess of chemical origin ingredients present on the label. So, techniques for the inclusion of other materials of animal origin such as hydrolyzed collagen, an emulsion of skins, and materials coming from the smooth muscles of different species are being developed, which are easy for consumers to read in the list of ingredients, as well as to improve flavor and add succulence to meat products (Bueno, 2008).

Collagen, being a product of animal origin, plays an important role in human diet due to its content of some essential amino acids and for its contribution to the prevention of some joint diseases (Sousa *et al.*, 2017). The addition of collagen in meat formulations can provide additional biological value and interesting sensory attributes for consumers (Neklyudov, 2003). In fact, this material has been incorporated into this type of derivatives in order to improve water holding capacity and increase the protein content of different formulations (Prestes *et al.*, 2012).

As part of this new trend to provide more "natural" foods called "Clean Labeling", there is the idea including healthier additives in the elaboration of York hams, without affecting the sensorial image the consumer has of this type of product (Tarte, 2009).

The objective of this research is to evaluate the effect of the total substitution of vegetable protein and phosphates for hydrolyzed collagen and citric fibers, respectively, on losses by cooking and syneresis of a chopped York ham, in order to achieve a product with partial clean labeling.

MATERIALS AND METHODS

Ham preparation

Two formulations of cooked cured ham with a 52.9% extender and water added were prepared from a standard formulation (Table 1). Polyphosphate was replaced by two different commercial citrus fibers, citrus fiber A and Citrus fiber B, whereas vegetable protein was replaced by dehydrated pork collagen. Citrus fiber A contains guar gum, different from citrus fiber B that contains commercial hydrolyzed collagen (commercial name ScanPro T92) as main component.

Meat preparation began with pH measurement to determine if there was a pale, soft and exudative meat (PSE); six different measurements were made in the different muscles that make up the leg. Once this defect was ruled out, meat was cut (equivalent to 80% of the total meat) in pieces of 50 g to be later tenderized twice with the JACCARD equipment. The remaining 20% of meat was passed through the 22-liter EG - 22 AI 1HP grinder and cut using an 8 mm disc. TECNAS Company provided the additives (including pork meat) mentioned in Table 1.

Table 1. Formulations used for ham preparation.

Ingredient	Formulation A	Formulation B	Control
		(%)	
Leg in pieces of 50g	56.87	56.87	48
Ground leg 8 mm disc	14.22	14.22	12
Hydrolyzed collagen	0.4	0.4	0
Ham flavoring	0.4	0.4	0.3
Sodium erythorbate	0.05	0.05	0.05
Colorant	0.025	0.025	0.03
Citrus fiber A	1	0	0
Citrus fiber B	0	1	0
Sodium lactate	1.9	1.9	2.5
Refined salt	1.25	1.25	1.2
Carrageenan	0.7	0.7	0.7
Potato starch	4	4	4
Curing salt	0.325	0.325	0.35
Ice/water	18.825	18.825	27
Milk protein	0	0	0.5
Vegetal protein	0	0	3
Sodium tripolyphosphate	0	0	0.4
Smoke powder	0	0	0.05

Initially, curing salt was incorporated into cold water by following the limits established by ICONTEC (Colombian Institute of Technical Standars) through NTC 1325, completely dissolved and then, refined salt was added. Subsequently, phosphates or citrus fibers A or B were added as appropriate. Color, sodium lactate, hydrolyzed collagen or vegetable protein were added to the water according to the formulation, ham flavoring, and finally, carrageenan and potato starch were mixed. After preparing the brine, it was incorporated along with the meat to the TORREY vacuum tumbler MV25, allowing the meat matrix to absorb all solids and liquids. Mechanical and vacuum mixing were carried out for 1 h, allowing to stand for 30 min; this process was performed twice. After this time, the dough was removed from the equipment and left in refrigeration at 3 °C for 24 h. Then, an additional mechanical blend was performed in a CI TALSA mixer with a capacity of 10 L for 15 min, after was taken to heat treatment.

For cooking, a CI TALSA kettle with a capacity of 200 L was used, with the aim of cooking by immersion for 2.5 h. Conditions were: hot water at a temperature not higher than 80 °C (but higher than the internal) and until reaching a core temperature of 72 °C. Once the cooking was finished, the cooked ham was removed from hot water, leaving it to rest for 2 h, subsequently, it was left in refrigeration at a temperature of 3 °C for 24 hours, reaching a final extension of 52.9%. Finally, the mass of processed ham was determined, chopped and packed in FE225557 vacuum plastic bags that fulfill the Colombian Technical Norm NTC171 (size 18×22 cm) with weights between 250 g and 280 g.

Syneresis evaluation

Samples were taken in triplicate for each treatment on days 0 and 10. Syneresis was measured by means of a SHIMADZU electronic balance, taking the initial weight of ham on day 0 and the final weight on day 10, after removing supernatant fluid. Syneresis results are presented as a percentage of its initial weight (Lowder *et al*., 2011; Prahbu, 2004).

Texture analysis

The analysis of compression force measurement was performed in a texture analyzer TA-XT2i (Stable Micro Systems). The Warner-Bratzler blade attachment was used with the following specifications: Pre-test speed 2.00 mm s^{-1}, test speed 2.00 mm s^{-1}, post-test speed 1.5 mm s^{-1}, with a distance of 40.0 mm and a cell of 50 kg. Samples of ham were cut into 4 cm wide by 10 cm long strips in order to carry out five repetitions for each treatment, looking for the maximum point of force exerted by the equipment for ham break (Prestes *et al*., 2012).

Sensory evaluation

A hedonic test was carried out with a panel of expert technicians composed of 16 people aged between 25 and 35 years, who were given three coded samples (M1 - control, M2 - citrus fiber A and hydrolyzed pork collagen, and M3 - citrus fiber B and hydrolyzed pork collagen) previously. They were asked to rate each of the products on a scale from 1 to 9, where 9 corresponded to "I like it very much," and 1 corresponded to "I dislike it a lot."

Statistical analysis

Fisher's test was implemented for analysis. A two-by-one factorial design was carried out, where phosphates were replaced by citrus fiber A and B, and vegetable protein by dehydrated pork collagen. This design was analyzed by statgraphics XVI statistical software, and ANOVA. Results were considered significant when $P<0.05$.

RESULTS AND DISCUSSION

Syneresis evaluation

Syneresis presented significant differences ($P<0.05$) in all treatments, as shown in Table 2. Phosphates, protein, and carrageenan help retain water in the first days of the manufactured ham, but over time, the links between hydrocolloids and meat protein become weak, letting the moisture out of the product. Treatments with citrus fiber A and B showed more stability in the water holding capacity because, once cooked and cooled, the hydrolyzed pork collagen forms a gel matrix able to trap free water strongly, as mentioned by Schilling *et al*. (2003) and Lowder *et al*. (2011) in their investigations.

Table 2. Syneresis in York hams made with and without vegetable protein and phosphates on day 10.

Sample	Initial weight	Final weight	Syneresis	Syneresis
	(g)			(%)
Control	269.33±5.13 a	263.00±5.57 a	6.33 a	2.41 a
Citrus fiber A	280.00±5.65 b	275.50±6.36 b	4.50 b	1.63 b
Citrus fiber B	244.33±8.02 c	241.67±8.08 c	2.67 c	1.10 c

Different letters in the same column denote significant differences between the York ham formulations, based on Fisher's test ($P<0.05$).

Aguilar (2011), in their study of a citrus fiber added to cooked ham, found that when replacing carrageenan with citrus fiber, it presented a greater capacity to interact with fibrillar structures of meat, forming a homogeneous network that traps high concentrations of water, being an efficient substitute for carrageenan in this type of meat product.

Data found in this study resemble those obtained by Resconi *et al.* (2016), who used rice and potato starches as partial phosphate replacements; they found that cooking loss was reduced in hams containing starches and minimal amounts of sodium tripolyphosphate. They also mentioned that the loss by cooking and positive acceptance by the sensory panel is achieved with the inclusion of 2% starch.

This study showed that when replacing polyphosphates with citrus fiber B, and vegetable protein with hydrolyzed pork collagen, there was greater moisture retention due to the low percentage of syneresis on day 10, compared to the other two treatments, thanks mainly to the interactions of these two ingredients with the specific meat proteins. The citrus fiber, a polysaccharide extracted from citrus peel, is used in meat products due to the technological functions it provides, such as water-holding, helping to improve the meat emulsion; it also reduces weight loss from cooking and dripping during the shelf life. Furthermore, the addition of fiber helps to preserve the juiciness of the product, and since the fiber flavor is neutral, it maintains the sensory properties of the product (Henning *et al.*, 2016; Kim and Paik, 2012; Mehta *et al.*, 2015; Verma and Banerjee, 2010). According to the above mentioned, it is demonstrated the superb performance and the correct interaction that is presented with myofibrillar proteins, allowing optimal results in the meat emulsion.

The hydrolyzed collagen of pork is a material obtained from different sources, among others, the skin. Its cohesive property is because of its chemical composition, which allows an interaction protein-protein with better performance. Studies have shown that using this material for better gel strength, helps retain more free water content over time (Schilling *et al.* 2003; Lowder *et al.* 2011).

Texture analysis

The three treatments have significant differences (P<0.05), being citric fiber B, the one that reaches higher values after the control. On day 0, the force in the control was 28.08 N and 21.35 N on day 10. Citrus fiber A showed an average force of 12.58 N and 16.02 N on days 0 and 10, respectively, while citrus fiber B reached 19.75 N and 15.56 N on day 0 and 10, approaching slightly more the data produced by the ham control. The results are shown in Table 3.

Table 3. Texture of hams made with and without phosphates and vegetable protein on days 1 and 10.

Samples	Day 1			Day 10		
	Force (N)	Distance (mm)	Time (s)	Force (N)	Distance (mm)	Time (s)
Control	28.08±2.14 a	4.65±0.08 a	4.65±0.08 a	21.35±2.94 a	4.62±0.11 a	4.62±0.11 a
Citrus fiber A	12.58±2.79 b	3.90±0.22 b	3.90±0.22 b	16.02±6.19 b	4.68±0.51 b	4.68±0.51 b
Citrus fiber B	19.75±4.34 c	4.20±0.11 c	4.20±0.11 c	15.56±6.12 c	3.92±0.29 c	3.92±0.29 c

Different letters in the same column denote significant differences between the York ham formulations, based on Fisher's test (P>0.05).

Citric fiber A presented a different behavior regarding the control and citric fiber B, showing a greater cutting force 10 days after the ham was elaborated, while control ham and the one containing citric fiber B decreased this parameter over time.

The components of citric fibers are hemicellulose, cellulose, pectin, lignin, oligosaccharides, gums, and waxes (Trowell *et al.*, 1985). Guar gum, a seed extract of *Cyamopsis tetragonolobus* (leguminous plant) (Prestes *et al.*, 2012), is a component of citrus fiber A. It can contribute to the water-holding, as well as improve texture in meat products; therefore, it allows establishing that the loss of humidity and force on day 10, is due to the presence and interaction of this gum in citrus fiber A.

In the present study, treatments with citrus fibers produced lower values than the control, with respect to the force of the attachment used to cut ham slices, an analysis that agrees with studies conducted by Han and

Bertram (2017) and Verma *et al.* (2010), which found a decrease in hardness of canned products added with dietary fibers. They informed that dietary fiber in meat products interrupts the formation of protein-water or protein-protein gel, thus decreasing the resistance of gel in meat products, leading to the conclusion that poor interaction between these compounds led to softening the texture (Choe *et al.*, 2018).

According to Pereira *et al.* (2011), the addition of hydrolyzed pork collagen in fine-grained products, allows the increase in water-holding, chemically immobilizing it through a protein matrix, which swells once it makes contact with water. In addition, collagen fibers and the cohesion of the dough contribute to the final product firmness (Sousa *et al.*, 2017). In similar studies, Choe *et al.* (2013) found how pork skin collagen together with wheat fiber influences hardness in fine-grained products because the hardness increases as collagen increases its presence in this type of meat, requiring more energy to chew it.

On the other hand, Resconi *et al.* (2015) showed that hardness, cohesiveness and gumminess decreased as rice starch was incorporated, what agrees with the present study. Previous investigations (Motzer *et al.*, 1998; Schilling *et al.*, 2003) have reported a reduction in hardness, chewiness and cohesion when including starch in cooked hams; these effects were attributed basically to the moisture retention capacity of starch. Also, as phosphates are incorporated, elasticity increases, while hardness and gumminess reduce their values. The presence of phosphates increases the ionic strength of the extraction solution, which favors, even more, the availability of fibrous proteins to fulfill different quality functions.

Sensory evaluation

Significant differences were found among the three treatments ($P<0.05$) regarding the acceptability by panelists (Figure 1). The treatment containing citrus fiber A obtained the lowest rating according to experts' perception, who described a residual flavor in the ham piece as well as some unpleasant aspects. The treatment with citrus fiber B, obtained higher scores in the evaluation of product acceptance, with pleasant attributes according to 60% of attendees to the test. When related to the applied texture test, this treatment presented greater force used by the equipment to cut in ham slices, characteristic similar to that described by experts when giving their appreciation about the texture of the product.

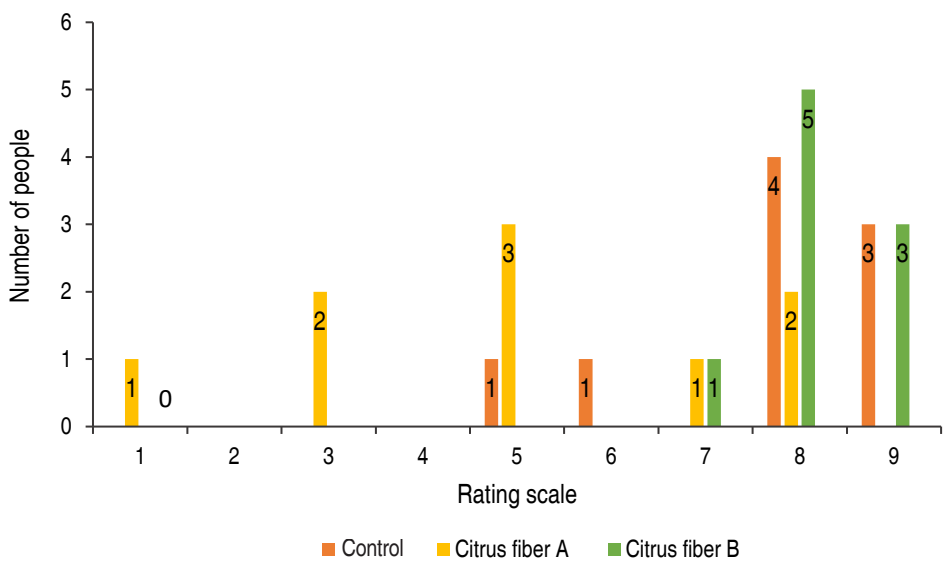


Figure 1. Sensory evaluation

In previous research, Youssef and Barbut (2011) found that Guar gum has the ability to reduce the concentration of myoglobin in meat, which is why products containing this ingredient in their formulation become more opaque, generating less acceptance in consumers.

Meanwhile, Sousa *et al*. (2017) showed similar values in the sensory test for Frankfurt sausages to which hydrolyzed collagen was applied; regarding the control, they did not differ in appearance, aroma and general acceptance when fat was substituted for hydrolyzed collagen, even when its highest concentration was incorporated. Méndez-Zamora *et al*. (2015) also replaced pork fat with collagen in sausages, found that said replacement had a greater effect on aroma and general acceptability, improving the formula of this product, concluding this way is a very interesting and viable alternative to reduce, not only fat but some other ingredients in different meat products.

For Tomaschunas *et al*. (2013), sausages to which citrus fiber was added have the potential to increase consumers' acceptability by comparing them with other products with similar characteristics, due to the interaction they generate with meat proteins, giving them a better texture. A result very similar to that obtained in the present study, where treatment with citrus fiber B (only citrus extracts), obtained the better qualification, even than the control.

In low-fat, dry-fermented sausages, orange-orange fiber had better results compared to cereal fibers and other fruits, with sensory scores similar to those of conventional sausages (García *et al*., 2002; Powell, 2017).

CONCLUSIONS

Results obtained in variables such as syneresis, sensory analysis and texture tests for treatments with citrus fiber A and B showed significant differences with the control (P<0.05), indicating that some of its components were not compatible with meat protein; the treatment with citrus fiber B presented acceptability in texture and sensorial attributes. The total replacement of the vegetable protein as well as phosphates in York hams generates an acceptable product to the consumer, showing sensory characteristics similar to those presented by a conventional product with chemical additives in its formulation. Citric fiber and hydrolyzed pork collagen allow starting partially clean labeling for this type of product. Further studies are required in order to eliminate other additives and thus establish total clean labeling.

REFERENCES

Aguilar JA. 2011. Efecto de la utilización de bagazo de naranja como extensor funcional sobre las propiedades fisicoquímicas y texturales de jamón cocido. Nacameh 5(2): 27–39. doi: 10.24275/uam/izt/dcbs/nacameh/2011v5n2/Aguilar

ANS - EFSA Panel on Food Additives and Nutrient Sources added to Food. 2010. Statement on nitrites in meat products. EFSA Journal 8(5): 1538. doi: 10.2903/j.efsa.2010.1538

Bueno RVCC. 2008. Efeito da fibra de colageno na qualidade funcional de "cooked frozen beef" (Tesis de maestria). Universidade Estadual de Campinas. Campinas, Brasil. 107 p. http://repositorio.unicamp.br/jspui/handle/REPOSIP/255342

Choe JH, Kim HY, Lee JM, Kim YJ and Kim CJ. 2013. Quality of frankfurter-type sausages with added pig skin and wheat fiber mixture as fat replacers. Meat Science 93(4): 849–854. doi: 10.1016/j.meatsci.2012.11.054

Choe JH, Lee JM, Jo K, Jo C, Song M and Jung S. 2018. Application of winter mushroom powder as an alternative to phosphates in emulsion-type sausages. Meat Science 143: 114–118. doi 10.1016/j.meatsci.2018.04.038

García ML, Dominguez R, Galvez MD, Casas C and Selgas MD. 2002. Utilization of cereal and fruit fibres in low fat dry fermented sausages. Meat Science 60(3): 227–236. doi: 10.1016/S0309-1740(01)00125-5

Han M and Bertram HC. 2017. Designing healthier comminuted meat products: Effect of dietary fibers on water distribution and texture of a fat-reduced meat model system. Meat Science 133: 159–165. doi: 10.1016/j.meatsci.2017.07.001

Henning SSC, Tshalibe P and Hoffman LC. 2016. Physico-chemical properties of reduced-fat beef species sausage with pork back fat replaced by pineapple dietary fibres and water. LWT 74: 92-98. doi: 10.1016/j.lwt.2016.07.007

Honikel KO. 2004. Curing Agents. pp. 195 - 201. In: Jensen WK, Devine C and Dikeman M (eds.). Encyclopedia of meats sciences. Second Edition. Elselvier, Oxford. 1712 p.

ICONTEC - Instituto Colombiano de Norma técnica. Industrias Alimentarias. Productos cárnicos procesados no enlatados. NTC 1325, quinta actualización. Colombia.

Kim HJ and Paik HD. 2012. Functionality and application of dietary fiber in meat products. Korean Journal for Food Science of Animal Resources 32(6): 695-705. doi: 10.5851/kosfa.2012.32.6.695

Lowder AC, Goad CL, Lou X, Morgan JB and Dewitt CAM. 2011. Evaluation of a dehydrated beef protein to replace sodium-based phosphates in injected beef strip loins. Meat Science 89(4): 491–499. doi: 10.1016/j.meatsci.2011.05.013

Mehta N, Ahlawat SS, Sharma DP and Dabur RS. 2015. Novel trends in development of dietary fiber rich meat products—a critical review. Journal of Food Science and Technology 52(2): 633-647.

doi: 10.1007/s13197-013-1010-2

Méndez-Zamora G, García-Macías JA, Santellano-Estrada E, Chávez-Martínez A, Durán-Meléndez LA, Silva-Vásquéz R and Quintero-Ramos A. 2015. Fat reduction in the formulation of frankfurter sausages using inulin and pectin. Food Science and Technology 35(1): 25–31. doi: 10.1590/1678-457x.6417

Moreno. 2013. Composición en grasa total y especialmente en ácidos grasos "trans" en productos de alimentación infantil y juvenil (Tesis de doctorado). Universidad Conplutense de Madrid, Madrid, España. 274 p. https://eprints.ucm.es/22406/

Motzer EA, Carpenter JA, Reynolds AE and Lyon CE. 1998. Quality of Restructured Hams Manufactured with PSE Pork as Affected by Water Binders. Journal of Food Science 63(6): 1007–1011. doi: 10.1111/j.1365-2621.1998.tb15843.x

Neklyudov AD. 2003. Nutritive Fibers of Animal Origin: Collagen and Its Fractions as Essential Components of New and Useful Food Products. Applied Biochemistry and Microbiology 39(3): 229–238. doi: 10.1023/A:1023589624514

Pereira AGT, Ramos EM, Teixeira JT, Cardoso GP, Ramos ALS and Fontes PR. 2011. Effects of the addition of mechanically deboned poultry meat and collagen fibers on quality characteristics of frankfurter-type sausages. Meat Science 89(4): 519–525. doi: 10.1016/j.meatsci.2011.05.022

Powell MJ. 2017. Physical and chemical effects of citrus fiber as a natural alternative to sodium tripolyphosphate in uncured all-pork bologna and oven-roasted turkey breast (Master's thesis). Iowa State University. Iowa, United States. 105 p. doi: 10.31274/etd-180810-5221

Prahbu GA, Doerscher DR and Hull DH. 2004. Utilization of Pork Collagen Protein in emulsified and whole muscle meat products. Journal of Food Science 69(5): C388-C392 doi: 10.1111/j.1365-2621.2004.tb10703.x

Prestes RC, Carneiro EBB and Demiate IM. 2012. Hydrolyzed collagen, modified starch and guar gum addition in turkey ham. Ciência Rural 42(7): 1307–1313. doi: 10.1590/s0103-84782012005000037

Resconi VC, Keenan DF, Gough S, Doran L, Allen P, Kerry JP and Hamill RM. 2015. Response surface methodology analysis of rice starch and fructo-oligosaccharides as substitutes for phosphate and dextrose in whole muscle cooked hams. LWT - Food Science and Technology 64(2): 946–958. doi: 10.1016/j.lwt.2015.06.053

Resconi VC, Keenan DF, García E, Allen P, Kerry JP and Hamill RM. 2016. The effects of potato and rice starch as substitutes for phosphate in and degree of comminution on the technological, instrumental and sensory characteristics of restructured ham. Meat Science 121: 127–134. doi: 10.1016/j.meatsci.2016.05.017

Restrepo CM y López DP. 2013. Embutidos: evolución y proyección de un mercado cambiante. En: Tecnas S.A., http://tecnas.com.co/embutidos-evoluci%C3%B3n-y-proyecci%C3%B3n-de-un-mercado-cambiante Consulta: agosto 2018.

Restrepo DA. 2018. Ingredientes y aditivos empleados en la industria cárnica: Nuevas alternativas. pp. 158-159. En: La carne: ciencia, tecnología y salud. Primera edición. Editores Publicidad S.A.S., Medellín. 440 p.

Schilling MW, Mink LE, Gochenour PS, Marriott NG and Alvarado CZ. 2003. Utilization of pork collagen for functionality improvement of boneless cured ham manufactured from pale, soft, and exudative pork. Meat Science 65(1): 547–553. doi: 10.1016/S0309-1740(02)00247-4

Sebranek JG. 2009. Basic curing ingredients. pp. 1 - 23. In: Tarté R (ed.). Ingredients in meats products: Properties, functionality and applications. Springer, New York. 432 p.

Sousa SC, Fragoso SP, Penna CRA, Arcanjo NMO, Silva FAP, Ferreira VCS, Barreto MDS and Araújo ÍBS. 2017. Quality parameters of frankfurter-type sausages with partial replacement of fat by hydrolyzed collagen. LWT - Food Science and Technology 76(Part B): 320–325. doi: 10.1016/j.lwt.2016.06.034

Tarte R. 2009. Ingredients in Meat Products: Properties, Functionality and Applications. Springer, New York. 419 p

Tomaschunas M, Zörb R, Fischer J, Köhn E, Hinrichs J and Busch-Stockfisch M. 2013. Changes in sensory properties and consumer acceptance of reduced fat pork Lyon-style and liver sausages containing inulin and citrus fiber as fat replacers. Meat Science 95(3): 629–640. doi: 10.1016/j.meatsci.2013.06.002

Trowell H, Burkitt D and Heaton K. 1985. Definitions of dietary fibre and fibre-deplered foods and disease. London: Academic press. Meat Sciense 157(2): 107883. doi: 10.1016/j.meatsci.2019.107883

Verma AK, Sharma BD and Banerjee R. 2010. Effect of sodium chloride replacement and apple pulp inclusion on the physico-chemical, textural and sensory properties of low fat chicken nuggets. LWT - Food Science and Technology, 43(4): 715–719. doi: 10.1016/j.lwt.2009.12.006

Verma AK and Banerjee R. 2010. Dietary fibre as functional ingredient in meat products: a novel approach for healthy living—a review. Journal of Food Science and Technology 47(3): 247-257. doi: 10.1007/s13197- 010-0039-8

WHO - World Health Organization. 2018. Noncommunicable diseases. In: http://www.who.int/es/news-room/fact-sheets/detail/noncommunicable-diseases Accessed: August 2018.

Youssef MK and Barbut S. 2011. Effects of two types of soy protein isolates, native and preheated whey protein isolates on emulsified meat batters prepared at different protein levels. Meat Science 87(1): 54–60. doi: 10.1016/j.meatsci.2010.09.002

Research article

http://www.revistas.unal.edu.co/index.php/refame

Revista Facultad Nacional de Agronomía

Substitution of *Canna edulis* starch for a mixture of potato/cassava starch in the production of almojábanas

Substitución de almidón de *Canna edulis* por una mezcla de almidón de papa/yuca en la elaboración de almojábanas

doi: 10.15446/rfnam.v73n3.83836

Stalin Santacruz[1,2]* and Gabriela Cobo[1]

ABSTRACT

Almojábana is a kind of food that varies in its composition depending on the geographical place where it is baked. In Ecuador, almojábana is based on cheese, wheat flour and *Canna edulis* starch. *C. edulis* planting has diminished, leading to a high cost of the starch and less availability on the market. The present work studied *C. edulis* starch substitution by a mixture of potato and cassava starches on the elaboration of almojábanas. Specific volume, crumb structure, textural properties and sensory analyses were used to find the best starch substitution. Mixtures of cassava and potato starch (25/75, 35/65, and 45/55) and starch dough resting time (10, 15, and 20 h) were investigated. Hardness, elasticity, chewiness, specific volume, average size cell, the number of cells per area and the total area of cells of almojábanas were determined. Results showed that a mixture of 25% potato, 75% cassava and 20 h resting time could substitute *C. edulis* starch in almojábana baking. The obtained almojábanas had different flavor compared to a control sample (based on *C. edulis* starch). Starch substitution reduced the cost of raw materials (starch) by 60%.

Keywords:
Crumb
Sensory evaluation
Specific volume
Starch mixture
Texture

RESUMEN

La almojábana es un alimento cuya composición varía dependiendo del lugar donde es elaborado. En Ecuador, se elabora con queso, harina de trigo y almidón de *Canna edulis*. El cultivo de *Canna edulis* ha disminuido dando como resultado un elevado costo del almidón y su menor disponibilidad en el mercado. En el presente trabajo se estudió la substitución de almidón de *C. edulis* por mezclas de almidones de papa y yuca en la elaboración de almojábanas. El volumen específico, estructura de la miga, propiedades de textura y análisis sensorial fueron utilizados para encontrar la mejor sustitución de almidón. Mezclas de almidones de papa y yuca (25/75, 35/65 y 45/55) conjuntamente con tiempo de reposo de las masas de almidón (10, 15 y 20 h) fueron estudiadas como variables del proceso. La dureza, elasticidad, masticabilidad, volumen específico, tamaño promedio de celda, número de celdas por área y área total de las celdas fueron los parámetros investigados. Los resultados mostraron que una mezcla de 25% papa y 75% yuca, y un tiempo de reposo de 20 min pudieron substituir al almidón de *C. edulis*. Las almojábanas obtenidas tuvieron sabor diferente en relación con el control de almidón de *C. edulis*. La substitución de este almidón redujo los costos de materia prima en un 60%.

Palabras clave:
Miga
Análisis sensorial
Volumen específico
Mezcla de almidones
Textura

[1] Universidad San Francisco de Quito. Av. Diego de Robles y Vía Interoceánica. CP 170901,Quito, Ecuador.
[2] Universidad Laica Eloy Alfaro de Manabí. Av. Circunvalación – Vía a San Mateo. CP 130804, Manta, Ecuador.
* Corresponding author: <stalin.santacruz@gmail.com>

Received: March 9, 2020; Accepted: July 17, 2020
Rev. Fac. Nac. Agron. Medellín 73(3): 9341-9347. 2020

ISSN 0304-2847 / e-ISSN 2248-7026

Almojábana is a baked food that its composition depending on the geographical place where it is elaborated. Therefore, almojábana may look and taste different due to differences in ingredients and processing. Almojábana is an Arab dessert based on cheese and wheat flour. Arabs spread the recipe to Spain, where it was modified and brought later to America (De la Granja Santamaría, 1970). In Ecuador, almojábanas have been produced since 1900 by small producers mainly for local consumption, with a recipe that is based on cheese, wheat flour and *Canna edulis* starch (Cobo, 2014).

C. edulis is a native plant from America. *C. edulis* rhizomes are used for starch extraction in tropical and subtropical countries (Caicedo *et al*., 2000). In Latin America, it is cultivated in Peru, Ecuador, Bolivia, Brazil, Venezuela, and Colombia (Caicedo *et al*., 2003). According to Caicedo *et al.* (2000). *C. edulis* crop has diminished due to the difficulty of the starch extraction process, pests and low economic profit compared to other crops. The low cropping has triggered high-cost *C. edulis* starch and less availability on the market (Caicedo *et al*., 2003). Therefore, there are economical and supplying problems for producers of foods based on this starch. Moreover, low planting may lead to the disappearance of a native crop, which has a promising starch for industrial applications (Parra-Huertas, 2012).

C. edulis starch is an important ingredient for the production of Ecuadorian almojábanas. *C. edulis* starch is also used to make food products like biscuits in Colombia (González, 2012). However, a expensive *C. edulis* starch with low availability has forced to look for starch substitutes.

C. edulis starch has a granule size between 35 and 101 µm, amylose content that varies from 25 to 45% (Yanuro, 2018) and a gelatinization temperature between 60 and 79 °C (Fonseca-Florido *et al*., 2016; Aprianita *et al*., 2014). Potato starch has granule size between 10 and 110 µm (Singh *et al*., 2003), amylose content that varies from 16 to 24% (Jane *et al*., 2010) and a gelatinization temperature between 56 and 67 °C. Cassava starch has a granule size between 5 and 45 µm and amylose content that varies from 14 to 19% (Gunaratne and Hoover 2002). Although cassava starch has different properties compared to *C. edulis*, it may be a good substitute since it has shown good results on the elaboration of baking products such as Pandebono (bread-based on fermented cassava starch) or *pão de queijo* (cheese bread, from Brazil), both with formulations very similar to almojábana. On the other hand, potato starch has similar properties to *C. edulis* starch, making potato and cassava starches proper choices for substitution (Salinas, 2014; Intriago and Muñoz, 2014) Both tuber starches are available on the Ecuadorian market. They have lower cost, 2,350 USD t^{-1} and 800 USD t^{-1}, respectively, compared to *C. edulis* starch (3,000 USD t^{-1}).

Therefore, the objective of this work was to study the substitution of *C. edulis* starch by a mixture of potato and cassava starch in the almojábanas making. The specific volume, crumb structure and textural properties (elasticity, chewiness, and hardness) were evaluated in the different almojábanas made with the starch mixtures. A weighing of the variables was used to obtain the best physical characteristics. Afterward, to find out differences between the best treatment and the control (based on *C. edulis* starch), sensory analyses were performed.

MATERIALS AND METHODS

Wheat flour was bought from La Moderna S.A. (Ecuador), *C. edulis* starch from an artisanal producer (Ecuador), potato starch from "Suiza Industrial del Ecuador" (Ecuador) and cassava starch from "Indumaíz del Ecuador" (Ecuador).

Almojábanas baking

Mixtures of potato and cassava starch were prepared according to Tables 1 and 2. Eggs were stirred in a kitchen mixer (Kitchen-Aid Professional 600, Mexico) at 220 rpm for 5 min, whereas cheese was stirred at 70 rpm for 4 min (Table 1).

Baking powder, wheat flour, starch or starch mixture (*C. edulis* for control sample) were blended and stirred at 50 rpm for 2 min. The formed dough was left at room temperature (approx. 16 °C) between 10 and 20 h (resting time). Afterward, milk was added to the dough to obtain a more liquid-like mixture. 70 g of the mixture was loaded to muffin pans and baked at 220 °C for 18 min. After baking, almojábanas were cooled before they were stored in polypropylene bags.

Table 1. Almojábana ingredients.

Ingredient	%[1]
Wheat flour	100
Starch/starch mixture[2]	100
Sugar	120
Fresh cheese	160
Baking powder	4
Eggs	100
Milk	40

[1] Percentage values are based on starch or starch mixture weight
[2] *C. edulis* starch or a mixture of potato and cassava starch

Table 2. Experimental design. Almojábanas elaboration with resting time and starch mixture composition as study factors

Starch mixture potato/cassava (%)	Resting time (h)	Treatment
25/75	10	A
	15	B
	20	C
35/65	10	D
	15	E
	20	F
45/55	10	G
	15	H
	20	I
C. edulis starch	16	Control

Specific volume and texture profile analysis

Specific volume was determined according to the AACC (2010) in triplicate. Texture profile analysis (TPA) was done according to Steffolani (2010). A texturometer (model CT3, Brookfield, USA) with a compression cell of 245 N, speed of 100 mm min^{-1}, maximum deformation of 40% and a diameter probe of 25 mm were used.

After baking, almojábanas were stored for 24 h before the TPA analyses. Measurements were done thrice testing the central part of the almojábana, which was cut with an electric knife (Oster 2619, U.S.A.). A sample of 25 mm depth, 52 mm height and 62 mm width, was loaded on the texturometer and compressed twice. Hardness, cohesiveness, and elasticity were measured, whereas chewiness was calculated according to equation 1.

$$\text{Chewiness}=\text{hardness}\times\text{cohesiveness}\times\text{elasticity} \quad (1)$$

Where:
Cohesiveness=A2/A1
Elasticity=L2/L1
A2: area under the second compression cycle of the TPA curve
A1: area under the first compression cycle of the TPA curve
L2: compression distance corresponding to the second compression cycle
L1: compression distance corresponding to the first compression cycle

Crumb structure

Crumb structure was determined according to Sciarini (2011). The average cell size, the number of cells per area and total area of cells (%) were determined in triplicate. Two softwares, Fiji and Peakfit (Systat Software, Inc.) were used (*Schindelin et al*., 2012). Fiji software was used to transform a crumb image into a gray color figure and afterward in a frequency histogram of a gray color scale (256 gray colors). Deconvolution of the frequency histogram was performed by Peakfit software. As a result, two Gaussian curves corresponding to the white and the black color. The intercept of the two curves was used as a limit, values under the intercept were appointed to the black color (crumb wall), and values above the intercept were associated with white color (crumb cell).

Experimental design

A Completely Randomized Design, 3^2+1, was used. Mixture of cassava and potato starch (25/75, 35/65 and 45/55) and resting time (10, 15 and 20 h) were used as factors (Table 2). A control sample was an almojábana, only baked with *C. edulis* starch. Hardness, elasticity, chewiness, specific volume, average size cell, number of cells per area and total area of cells were determined.

The composition of the starch mixture (Table 2) was decided based on previous assays. A substitution with potato starch above 60% produced an almojábana with low moisture and low elasticity. In contrast, a substitution with cassava with values above 80% produced an almojábana with a different flavor and higher elasticity compared to the control sample (almojábana based on *C. edulis* starch).

An ANOVA and a Tukey test with a significance level of 5% were applied by using IBM SPSS Statistics 20 software.

Sensory evaluation

The treatments with the best physical and textural characteristics were used to perform a triangle test. In this test, three samples were presented to the panelists simultaneously. Two of them were the same and one was different, the latter being the control sample. The panelist was asked to identify the different sample (Hernández, 2005).

The number of panelists was decided regarding three parameters, α=0.05 (moderate evidence), β=0.1 (90% confidence) and Pd=20% (20% of the population can detect difference among samples).

RESULTS AND DISCUSSION

Specific volume and texture profile analysis

Specific volume showed statistical difference among treatments (P<0.05). Resting time was the only significant factor. According to Table 3, treatments similar to control sample corresponded to group 1, with specific volumes between 1.75 and 2.09 $cm^3 g^{-1}$.

Table 3. Specific volume of the different treatments

Treatments	Specific volume ($cm^3 g^{-1}$)		
	Group, α = 0.05		
	1	2	3
Control	1.75		
I	1.81		
C	1.95	1.95	
E	1.96	1.96	
F	1.97	1.97	1.97
B	1.99	1.99	1.99
A	2.02	2.02	2.02
H	2.09	2.09	2.09
D		2.26	2.26
G			2.36

A high specific volume is usually required for baking products; however, almojábanas had a low specific volume. Almojábanas are prepared with similar ingredients than *pão de queijo*; it has a high amount of starch, cheese and eggs, leading to the formation of heavy dough and, therefore, a food product with low specific volume (Zavareze *et al.*, 2009).

Hardness

Almojábana hardness results are shown in (Table 4). Time of rest was the only factor that influenced almojábana hardness. Results showed that the treatment prepared with 25% potato, 75% cassava and 20 h resting time had a hardness of 21.29 N and was statistically similar to the control (23.80 N). Both samples had higher hardness compared to other samples (P<0.05). Cueto *et al.* (2011) found that high levels of cassava flour can generate pancakes and cakes with low hardness.

Different results may be due to the use of single starch or a starch mixture and the type of cheese on the elaboration of almojábana. Indeed, Pereira (1998) found that the type of cheese is responsible of the taste and texture of *pão de queijo*.

Table 4. Hardness and chewiness properties

Treatment	Hardness (N)			Treatment	Chewiness (N mm)		
	Group, α=0.05				Group, α=0.05		
	1	2	3		1	2	3
A	14.7			G	41		
G	15.7			B	42.3	42.3	
B	16.2			H	43.7	43.7	
D	16.5			I	44.7	44.7	
H	17.7	17.7		A	44.9	44.9	
E	18.6	18.6		F	46.4	46.4	46.43
I	18.7	18.7		E	48.7	48.7	48.73
F	18.9	18.9		D	51.3	51.3	51.3
C		21.3	21.3	C		56.3	56.3
Control			23.8	Control			61

Chewiness

Chewiness results showed differences among treatments. Four treatments were statistically comparable to the control (Table 4), treatment C (25% potato/75% cassava – 20 h), D (35% potato/65% cassava – 10 h), E (35% potato/65% cassava – 15 h) and F (35% potato/65% cassava – 20 h) with chewiness between 46.43 and 61.00 N mm.

Cauvain (2016) showed that changes in chewiness during storage are associated with starch retrogradation. On the other hand, Cuikeisha and Roberts (2009) showed that chewiness decreased proportionally to the quantity of fiber present. Knowing that retrograded starch behaves as fiber (Saaman, 2017), in the present work, mixtures of potato and cassava starch may promote a formation of retrograded starch in different amounts. It could affect the chewiness of the almojábana.

Elasticity

There was no statistical difference among treatments and control sample (P<0.05). However, almojábanas elaborated with long resting times (15 and 20 h) had a lower elasticity. Therefore a low capacity to recover the original size after compression, compared to almojábanas with resting times of 10 h. The control sample had elasticity analogous to almojábanas elaborated with 15 h resting time.

Previous study (Cueto *et al.*, 2011) showed that a high content of cassava starch, the cell size increased. This research showed that almojábanas with the highest content of cassava starch (treatments A, B and C) had

cell sizes with intermediate values. Different results could be due to the different ingredients used on food elaboration. The number of cells per area and total area of cells showed no statistical differences among treatments.

Crumb characterization

Cell size showed only dependence of the interaction between starch mixture and resting time. Treatment D had the largest cell size (5.61 mm^2), whereas treatment E had the smallest one (1.75 mm^2) (Table 5). Treatments corresponding to group 2 had cell sizes between 1.75 and 4.17 mm^2 and were equal to the control (P<0.05).

In order to obtain a treatment with the best physical characteristics, a weighing of the variables was performed by giving values as follows, 1 for cell size, 2 for specific volume, 3 for hardness and 4 for chewiness. Treatment C (25% potato/75% cassava and 20 h resting time) had the highest score (10 points), and, therefore, it was selected as the best treatment. Moreover, using this treatment, the cost of raw materials (starch) was reduced by about 60%.

Table 5. Almojábanas cell size

Treatments	Cell size (mm^2)	
	Group, α = 0.05	
	1	2
E	1.75	
B	2.03	2.03
G	2.47	2.47
I	2.48	2.48
Control	2.73	2.73
F	2.75	2.75
A	2.78	2.78
C	3.6	3.6
H	4.17	4.17
D		5.61

Sensory evaluation

In order to know if treatment C had the same characteristics as the control sample, a triangle test was executed. 39 was the number of correct answers to establish a significative difference (Hernández, 2005). A total of 46 panelists correctly identified the different samples, finding that treatment C had a texture similar to the control sample. As an additional comment, panelists evidenced differences between the almojábanas made with a mixture of potato/cassava starch and *C. edulis* starch.

CONCLUSIONS

The use of a starch mixture, potato (25%) and cassava (75%) for 20 h resting time to elaborate almojábana showed no differences compared to a control sample based on *C. edulis* starch. Treatment C could be reduced the cost of raw materials (starch) by 60%. Elasticity, chewiness and hardness were not affected by the starch substitution, whereas analysis of the crumb showed that cell size was affected by the starch substitution and the resting time.

REFERENCES

AACC. 2010. American Association of Cereal Chemist**.** International Approved Method of Analysis. 11th ed. AACC International, St. Paul.

Aprianita A, Vasiljevic T, Bannikova A and Kasapis S. 2014. Physicochemical properties of flours and starches derived from traditional Indonesian tubers and roots. Journal of Food Science and Technology 51: 3669-3679. doi: 10.1007/s13197-012-0915-5

Caicedo G, Rozo L y Bonilla U. 2000. La achira su producción y beneficio. CORPOICA, Bogotá. 51 p.

Caicedo G, Rozo L y Rengifo G. 2003. La achira. Alternativa agroindustrial para áreas de economía campesina. CORPOICA, Bogotá. 85 p.

Cauvain S. 2016. Bread and Other Bakery Products. pp. 431-

459. In: Subramaniam P (ed.). The Stability and Shelf Life of Food. Second Edition. Elsevier, Cambridge. 612 p. doi: 10.1016/B978-0-08-100435-7.00015-0

Cobo G. 2014. Substitución de almidón de achira (*Canna edulis*) por una mezcla de almidones de papa (*Solanum tuberosum*) y yuca (*Manihot esculenta*) para la elaboración de quesadillas (Tesis de pregrado). Universidad San Francisco de Quito. Quito, Ecuador. 74 p.

Cueto D, Pérez E, Borneo R y Ribotta P. 2011. Efecto de la adición de harina de yuca (*Manihot esculenta* Crantz) sobre las características sensoriales, reológicas y físicas de tortas y panquecas. Revista de la Facultad de Agronomía UCV 37: 64-74.

Cuikeisha S and Robert T. 2009. Dietary Fiber: Fulfilling the Promise of Added-Value Formulations. In: Kasapis S, Norton IT and Ubbink JB (eds.). Modern Biopolymer Science: Bridging the divide between fundamental treatise and industrial application. First Edition. Academic Press, Cambridge. 640 p. doi: 10.1016/B978-0-12-374195-0.00013-6

De la Granja Santamaría. 1970. Fiestas Cristianas en el Andalus II, Al-Andalus, XXXV. 124 p.

Fonseca-Florido H, Méndez-Montealvo G, Velazquez G and Gómez-Aldapa C. 2016. Thermal study in the interactions of starches blends: Amaranth and achira. Food Hydrocolloids 61: 640-648. doi: 1001016/j.foodhyd.2016.06.027

González G. 2012. Desarrollo de productos con alto contenido de almidón para la industria de alimentos. Fundación Universitaria Agraria de Colombia, Bogotá. pp. 1-51.

Gunaratne A and Hoover R. 2002. Effect of heat–moisture treatment on the structure and physicochemical properties of tuber and root starches. Carbohydrate Polymers 49(4): 425-437. doi: 10.1016/S0144-8617(01)00354-X

Intriago M and Muñoz G. 2014. Estudio de Factibilidad para la creación de una Empresa que produzca almidón de yuca, como materia prima para el mercado de Guayaquil. (Tesis de pregrado). Universidad Católica de Santiago de Guayaquil. Guayaquil, Ecuador. 102 p.

Jane J, Maningat C and Wongsagonsup R. 2010. Starch characterization, variety and application. In: Singh B (ed.). Crops and Uses. CAB, Chippenham. 528 p.

Parra-Huertas R. 2012. Physical-chemical and sensory characterization of compote from gulupa (*Passiflora edulis*), starch sagú (*Canna edulis*) and stevia. Vitae 19: S219-S221.

Pereira A. 1998. Factores que afetam a qualidade do pão de quiejo. CETEC, Belo Horizonte. 52 p.

Saaman R. 2017. Dietary fiber for the prevention of cardiovascular disease: fiber's interaction between gut microflora, sugar metabolism, weight control and cardiovascular health. Academic Press, London. 170 p.

Salinas L. 2014. Modelo de negocio para una empresa productora de almidón de papa de la provincia de Tungurahua. (Tesis de maestría). Escuela Politécnica Nacional, Quito, Ecuador. 227 p.

Sciarini L. 2011. Estudio del efecto de diferentes aditivos sobre la calidad y la conservación de panes libres de gluten. (Tesis doctoral). Universidad Nacional de la Plata. La Plata, Argentina. 208 p.

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez JY, White DJ, Hartenstein V, Eliceiri K, Tomancak P and Cardona A2012. open-source platform for biological-image analysis. *Nature Methods 9: 676-682. doi:* 10.1038/nmeth.2019

Singh N, Singh J, Kaur L, Sodhi N and Gill B. 2003. Morphological, thermal and rheological properties of starches from different botanical sources. Food Chemistry 81(2): 219-231. doi: 10.1016/S0308-8146(02)00416-8

Steffolani, M. 2010. Efecto de las enzimas pentosanasa, glucosa oxidasa y transglutaminasa en productos de panificación. (Tesis doctoral). Universidad Nacional de la Plata. La Plata, Argentina. 240 p.

Yanuro N. 2018. Evaluación de las propiedades físicoquímicas, térmicas y microestructurales del almidón de achira (*Canna edulis*). (Tesis doctoral). Universidad Nacional de Colombia, Colombia. 133p

Zavareze ER, Storck CR and Pereira J. 2009. Elaboração de pão de queijo com substituição do amido de mandioca por amido de batata-doce (*Ipomoea batatas*) submetido a diferentes processos de secagem. Brazilian Journal of Food Technology 12: 68-76. doi: 10.4260/BJFT20094908

Research article

http://www.revistas.unal.edu.co/index.php/refame

Revista Facultad Nacional de Agronomía

Gypsum incubation tests to evaluate its potential effects on acidic soils of Colombia

UNIVERSIDAD NACIONAL DE COLOMBIA

Pruebas de incubación con yeso para evaluar sus potenciales efectos en suelos ácidos de Colombia

doi: 10.15446/rfnam.v73n3.85259

Jorge Enrique Cuervo-Alzate[1]* and Nelson Walter Osorio[1]

ABSTRACT

Tropical soils are characterized by acidity and poor plant nutrient availability, limiting their agricultural productivity. These soils are commonly amended with lime, but its low solubility impairs its effectiveness to enhance soil fertility. The use of gypsum has gained attention among farmers due to its higher solubility and mobility in the soil, local accessibility, and low price. Therefore, this study was conducted to determine the effects of Agricultural Gypsum (AG) addition on ten Colombian acid soils that had poor fertility and contrasting their physical and chemical characteristics. Surface (0-20 cm) soil samples were air-dried, sieved (<2 mm), and transferred into plastic vases, 40 g (dry base) per vase. Increasing rates of gypsum were added by duplicate: 0.0, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0 g kg^{-1}. Then, the soils were incubated for two weeks and watered to maintain 50% of their maximum water holding capacity. Soil pH, Al^{+3}, Ca^{+2}, Mg^{+2}, K^{+}, $S\text{-}SO_4^{-2}$, and $P\text{-}H_2PO_4^{-2}$ were measured using standard methods. The results showed that AG addition significantly (P<0.05) increased soil exchangeable Ca^{+2}-K^{+}, Ca^{+2} saturation, $S\text{-}SO_4^{-2}$ concentration, and exchangeable Al^{+3}, particularly with doses above 4.0 g kg^{-1}. In contrast, soil Al^{+3} saturation, $P\text{-}H_2PO_4^{-2}$ and pH significantly decreased as the AG doses increased, while soil exchangeable Mg^{+2} levels were not significantly affected. The use of gypsum incubation tests could be promissory for its effects on soil amelioration associated mainly to increase soil exchangeable Ca^{2+} and $S\text{-}SO_4^{2-}$ and to decrease Al^{3+} saturation.

Keywords:
Acidity
Agricultural gypsum
Aluminum
Calcium sulfate
pH
Soil fertility

RESUMEN

Los suelos tropicales son característicamente ácidos y pobres en nutrientes, lo cual limita su productividad agrícola. Estos suelos usualmente son tratados con cal, pero su baja solubilidad limita su efectividad para mejorar la fertilidad. El uso de yeso agrícola ha ganado mucha atención debido a su mayor solubilidad y movilidad en el suelo, disponibilidad local y bajo precio. Por tanto, se realizó un estudio para determinar tales efectos en diez suelos acidos Colombianos con baja fertilidad y diferentes características físicas y químicas. Muestras de estos suelos (0-20 cm) se secaron al aire, tamizaron (<2 mm) y transfirieron a vasos plásticos. Dosis crecientes de yeso se aplicaron por cuadruplicado: 0,0; 0,25; 0,5; 1,0; 2,0; 4,0; 8,0 y 16,0 g kg^{-1}. Los suelos se humedecieron para mantenerlos a 50% de su máxima capacidad de retención de agua durante dos semanas. Se midió pH, Al^{+3}, Ca^{+2}, Mg^{+2}, K^{+}, $S\text{-}SO_4^{-2}$ y $P\text{-}H_2PO_4^{-2}$ usando métodos estandarizados. Los resultados muestran que al adicionar yeso agrícola se incrementó significativamente (P<0,05) los niveles de Ca^{+2}-K^{+} intercambiable, saturación de Ca^{+2} , $S\text{-}SO_4^{-2}$ y Al^{+3} intercambiable, particularmente con dosis mayores de 4,0 g kg^{-1}. En contraste, con el incremento de la dosis de yeso agrícola disminuyeron significativamente la saturación de Al^{+3} , y el pH; los niveles de Mg^{+2} no fueron significativamente afectados. El uso de las pruebas de incubación de yeso agrícola es promisorio para detectar mejoras en el suelo a través del incremento en los niveles de Ca^{2+} y $S\text{-}SO_4^{2-}$ y la disminución de la saturación de Al^{3+}.

Palabras clave:
Acidez
Yeso agrícola
Aluminio
Sulfato de calcio
pH
Fertilidad del suelo

[1] Facultad de Ciencias. Universidad Nacional de Colombia. AA 3840 Medellín, Colombia.
* Corresponding author: <cuervojorge11@gmail.com>

Received: May 15, 2020; Accepted: August 24, 2020
Rev. Fac. Nac. Agron. Medellín 73(3): 9349-9359. 2020

ISSN 0304-2847 / e-ISSN 2248-7026

About 25-30% of soils are classified as acidic and are located in some of the most important regions of food production around the world (Havlin *et al*., 1999). In Colombia, about 80% of the soils have this condition and, at least 50%, have problems of toxicity by aluminum (IGAC, 2015). It is one of the biggest limitations of crop production in acidic mineral soils. These soils are generally located in areas of high rainfall where potatoes, coffee, vegetables, corn, rice, fruit trees, and pastures are cultivated. Besides, these soils have problems associated with low base saturation, low pH, and intense leaching that may interfere with root growth and functioning (Van Raij, 2008). Amendments such as lime, dolomite limes, oxides and hydroxides of magnesium and calcium have been traditionally used to decrease the negative impacts of acidity. In addition, other materials, such as agricultural gypsum or combinations and silicates, are used in less proportion (Castro and Gómez, 2013).

Gypsum comes from Gypsites. It is the most common of the calcium sulfates; generally, it is found in secondary deposits, associated with $CaSO_4$ anhydrite or $CaSO_4 \cdot 1/2 H_2O$ bassanite (Van Raij, 2008; Osorno, 2012). Agricultural gypsum (AG), whose main component is calcium sulfate dihydrate ($CaSO_4 \cdot 2H_2O$), provides Ca (17-27%) and S (14-18%). Phosphogypsum is a by-product of the phosphate industry, where the dissolution of phosphate rock with sulfuric acid generates phosphoric acid and phosphogypsum (Fisher, 2011; Zapata, 2014; Saadaoui, 2017). It has a solubility of 2.5 g L^{-1}, being 200 times more soluble than agricultural lime and calcium carbonate (0.013 g L^{-1}) (Fisher, 2011; Zapata, 2014). This property allows it to move more towards subsurface horizons, where it is possible to precipitate aluminum (Van Raij, 2008). Phosphogypsum can be used as a direct source of Ca^{2+} or $S\text{-}SO_4^{2-}$ to improve plant growth in sodium-saline soils and reduce subsoil acidity (Prochnow *et al*., 2016), achieving greater presence of Ca^{2+} and less of Al^{3+} in the complex of change in depth, neutralizing its excess (Takahashi *et al*., 2006; Kalinitchenko and Nosov, 2019).

Due to the misconception that gypsum has a potential acidifying effect, incorrectly attributed to sulfate ion, and inducing cation leaching increase (i.e., Ca^{2+}, Mg^{2+}, and K^+), its use is not extensively recommended. However, some literature references support the benefits of using gypsum in agriculture. It is more soluble than lime, can be applied to the soil surface and percolated, breaking the chemical barrier of acidity in the subsoil imposed by the relative excess of Al^{3+}, contributes Ca^{2+} to the soil solution and also provides $S\text{-}SO_4^{2-}$ (Van Raij, 2008; Fisher, 2011; Castro and Gómez, 2013; Ramos *et al*., 2013; Prochnow *et al*., 2016; Favarin *et al*., 2018; Osorio, 2018; Kalinitchenko and Nosov, 2019).

In this study, a laboratory test was used to evaluate the potential effects of gypsum application on different soil fertility parameters of acidic soils in Colombia.

MATERIALS AND METHODS

Soils

Ten acidic soils of Colombia (Figure 1) were chosen based on following characteristics: pH $\leq$ 5.0, exchangeable aluminum $\geq$ 0.5 $cmol_c\ kg^{-1}$, calcium $\leq$ 2.0 $cmol_c\ kg^{-1}$, sulfur $\leq$ 6.0 mg kg^{-1}, sum of bases ($Ca^{2+}+Mg^{2+}+K^+$)<5.0 $cmol_c\ kg^{-1}$ (Gómez *et al*., 1991; Castro and Gómez, 2013; IGAC, 2014, 2015). These soils belong to diverse soil orders: Andisols, Entisols, Inceptisols, Ultisols, and Oxisols, as described in Table 1. The soils were named by the site/town where they were collected. Some selected characteristics of climate and soil taxonomy of these sites are shown in Table 1.

Fifteen subsamples were taken in an area of approximately 100 m^2 of each site of soil collection (Brown, 1987; Havlin *et al*., 1999; Sadeghian and Lynce, 2014). The subsamples were removed from the surface A horizon (0-20 cm) with a Dutch Auger and mixed thoroughly. 1 kg from each site was bagged, properly labeled, and sent to the soil laboratory of the Universidad Nacional de Colombia in Medellín (6°15' N, 75°35' W, 1495 masl, 22 °C). According to Osorio (2018), the soil samples were air-dried gradually for a week and passed through a 2-mm mesh sieve. The following laboratory methods were used (Osorio, 2018): pH in water 1:2 (w:v) with a calibrated pH meter; Ca, Mg, K content ($cmol_c\ kg^{-1}$) with 1 M ammonium acetate and analytical determination by atomic absorption; exchangeable aluminum ($cmol_c\ kg^{-1}$) extracted with 1 M KCl and quantified by titration; $S\text{-}SO_4^{-2}$ was extracted with 0.008 M calcium phosphate and quantified by turbidimetry; soluble P concentration extracted with 0.01 M $CaCl_2$ and quantified by the molybdate-blue method.

Figure 1. Map of Colombia with the location of the samples.

Table 1. General information on the sites and soils selected for this study.

Site	Department	Altitude (m)	Annual precipitation (mm)	Soil Taxonomy		Soil moisture regime	Soil temperature regime	OC (%)	Clay (%)	Sand (%)
				Order	Grand group					
La Tebaida	Quindio	1194	1850	Andisol	Hapludand	Udic	Isothermic	3.49	7	66
Anserma	Caldas	1784	2150	Andisol	Melanudand	Udic	Isothermic	3.82	16	54
Frontino	Antioquia	1385	2000	Andisol	Hapludand	Udic	Isothermic	1.80	18	61
Libano	Tolima	1521	2300	Andisol	Melanudand	Udic	Isothermic	6.68	5	55
Palestina	Caldas	1545	2500	Andisol	Melanudand	Udic	Isothermic	5.17	33	45
Palermo	Huila	1900	1700	Entisol	Troporthent	Udic	Isothermic	7.04	22	58
Manizales	Caldas	1348	1900	Inceptisol	Dystrudept	Udic	Isothermic	1.47	26	49
Zapatoca	Santander	1586	1700	Inceptisol	Dystrupept	Udic	Isothermic	1.22	5	54
Caucasia	Antioquia	77	2500	Ultisol	Paleoudult	Udic	Isohyperthermic	0.18	30	38
Carimagua	Meta	650	2400	Oxisol	Kandiustox	Ustic	Isohyperthermic	0.80	14	54

* Sources: Baldión and Guzman (2008, 2009), González (2013), IGAC (1995, 2015), Madero (2013), USDA (2010).
OC: Organic Carbon.

Incubation test

The Agricultural Gypsum used in this experiment was a by-product generated by the acidulation of a phosphate rock, a primary source of P for Monómeros Colombo Venezolanos S.A. Company (Barranquilla, Colombia), manufacture of compound fertilizers. The treatments consisted of the addition of increasing doses of this gypsum (0.0, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0 g kg^{-1})), which is roughly equivalent to 0, 500, 1.000, 2.000, 4.000, 8.000, 16.000 and 32.000 kg ha^{-1}. This method was adapted from a method proposed by Uchida and Hue (2000) and Ernani *et al.* (2006). Briefly,

this consisted of transferring 40 g of soil (dry weight) previously sieved (<2 mm) to 100 cm^3 plastic cups. Then, the respective dose of gypsum was added into the soil and mixed thoroughly.

The soil samples amended were watered to maintain 50% of the maximum water retention capacity (equivalent to field capacity) and left them in incubation for two weeks at room temperature (22 °C). Once the soils were dried gradually for a week, they were rewetted and dried again for another week. Then, some selected soil fertility parameters (pH, Ca^{2+}, Mg^{2+}, K^{+}, Al^{3+}, P-$H_2PO_4^-$, S-SO_4^{-2}) were measured using the methodologies described above.

Experimental design and statistics analysis

The incubation of each of the soils was considered as a separate experiment. The experimental design was completely randomized. The treatments consisted of eight doses of AG (including the unamended control); each treatment had four replications. The data were subjected to analysis of variance to evaluate the significant effects of treatments. Duncan's test of multiple ranges was used for mean separation. In both tests, a significance level $P<0.05$ was employed. The statistical analysis was made with the SAS software, version 9.0.

RESULTS AND DISCUSSION

The significant tendency to decrease pH with the increase of AG doses in all soils was evidenced. The variation of pH values (Δ mean, initial pH-final pH) between the control and the highest dose (16.0 mg kg^{-1}) was 0.36 and 0.86 units, depending on the type of soil (Table 2). Significant differences of Al^{3+} were detected in the soils with the treatments compared to the control (Table 3). As the AG doses increased, the exchangeable Al^{3+} content significantly ($P<0.05$) increased; however, no significant differences were detected with doses lower than 4.0 g kg^{-1}. This behavior was not similar to the pH, where a decrease was observed with the lowest dose (0.25 g kg^{-1}). It may be due to high concentrations of salts that could increase Al^{3+} concentrations. Salt cation replaces some of the interchangeable Al^{3+}, which is hydrolyzed in the solution, reducing the pH (Von Uexkull, 1986; Havlin *et al*., 1999). It could also be associated with the formation of compounds with very low solubility, and in the precipitation reaction, H^+ is released. Without neutralization by other components, it can cause a small decrease in soil pH (Zapata, 2014). However, the Al^{3+} saturation had a different behavior (Figure 2), which depend on the soil type.

Table 2. Soil pH after two weeks of incubation with AG.

AG doce (g kg^{-1})	Soil pH									
	La Tebaida	Anserma	Frontino	Líbano	Palestina	Palermo	Manizales	Zapatoca	Caucasia	Carimagua
0.0	5.07 a	5.16 a	3.94 a	5.13 a	5.14 a	4.84 a	4.38 a	4.64 a	4.78 a	4.70 a
0.25	4.94 b	5.03 bc	3.79 ab	5.07 ab	5.08 ab	4.72 b	4.23 b	4.51 b	4.74 ab	4.68 a
0.5	4.91 b	5.08 ab	3.87 a	5.10 a	5.06 ab	4.63 bc	4.19 c	4.39 c	4.70 b	4.46 b
1.0	4.89 b	4.94 cd	3.82 ab	5.00 b	4.99 bc	4.55 cd	4.06 d	4.35 cd	4.59 c	4.40 b
2.0	4.78 c	4.94 cd	3.64 bc	4.86 c	4.90 c	4.57 cd	3.97 e	4.30 de	4.49 d	4.19 c
4.0	4.71 c	4.84 d	3.55 c	4.49 e	4.67 e	4.57 cd	3.89 f	4.22 ef	4.39 e	4.13 cd
8.0	4.62 d	4.68 e	3.45 cd	4.60 d	4.61 e	4.52 d	3.86 f	4.16 f	4.27 f	4.06 d
16.0	4.55 d	4.59 e	3.26 d	4.42 e	4.78 d	4.31 e	3.73 g	4.06 g	4.07 g	3.84 e
Δ mean	0.52	0.57	0.68	0.71	0.36	0.53	0.65	0.58	0.71	0.86

Different lowercase letters within a column are significantly different ($P<0.05$), according to the multiple range Duncan test.
Δ mean: difference between the smallest and largest value

In some cases, Al^{3+} was significantly reduced, and it was most noticeable with the higher AG doses. The soils of Anserma, Libano, and Palermo soils exhibited low levels of Al^{3+} saturation (<15%) in their respective controls. Therefore, it was expected to have weaker effects with the AG treatments.

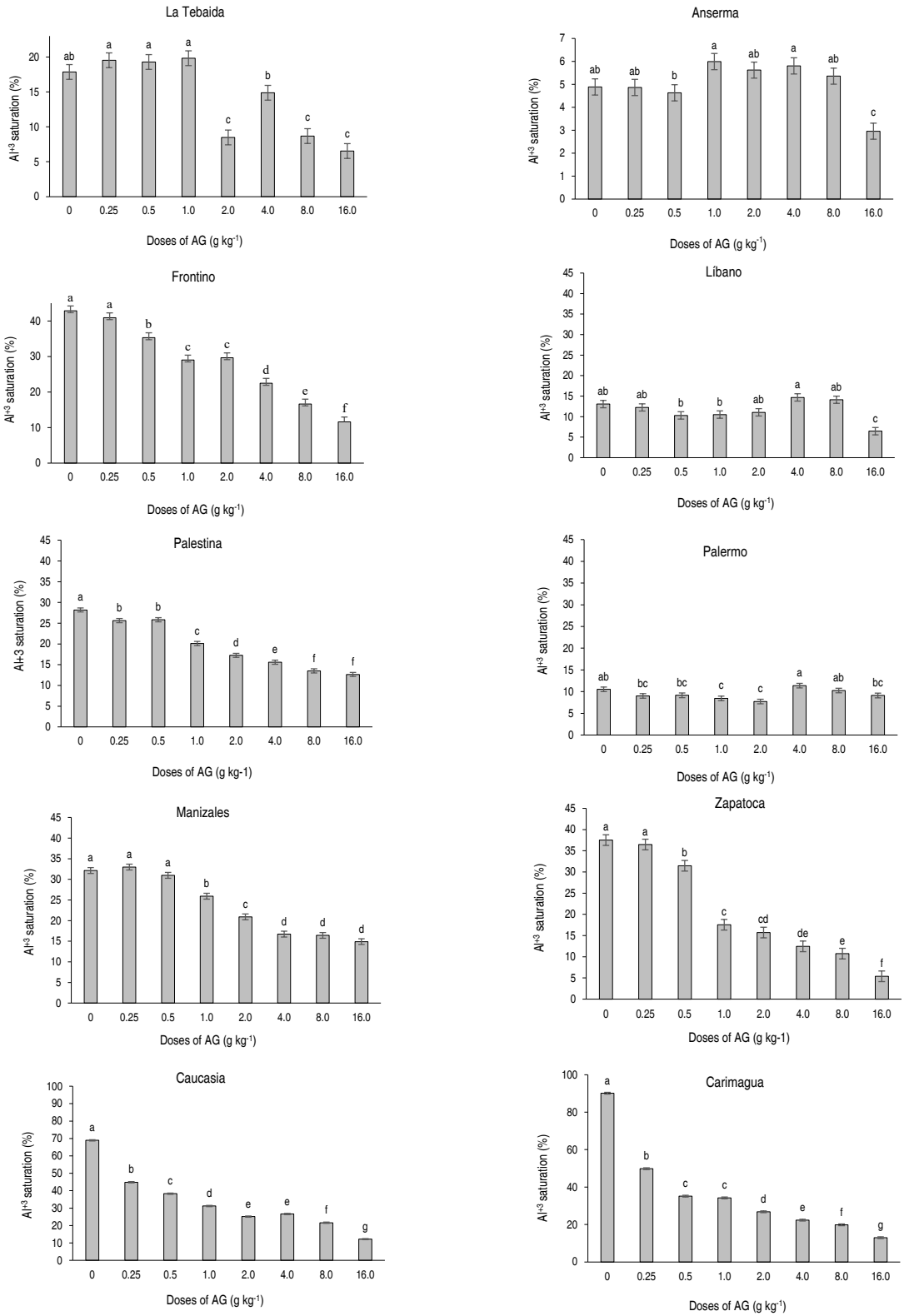


Figure 2. Soil Al^{3+} saturation (%) after two weeks of incubation with AG. Columns with different lowercase letters mean significant differences (P<0.05), according to Duncan test.

Table 3. Soil exchangeable Al^{3+} after two weeks of incubation with AG.

AG dose (g kg^{-1})	Soil exchangeable Al^{3+} ($cmol_c$ kg^{-1})									
	La Tebaida	Anserma	Frontino	Líbano	Palestina	Palermo	Manizales	Zapatoca	Caucasia	Carimagua
0.0	0.58 d	0.64 de	1.60 a	0.80 b	1.79 cd	0.89 b	1.78 f	1.51 de	2.23 ef	1.83 g
0.25	0.65 cd	0.58 de	1.83 a	0.83 b	1.70 d	0.84 b	1.91 de	1.40 e	2.16 f	1.86 fg
0.5	0.71 cd	0.55 e	1.75 b	0.79 b	1.83 bc	0.79 b	1.88 ef	1.61 cd	2.25 ef	1.93 f
1.0	0.83 c	0.73 cd	1.68 c	0.88 b	1.86 bc	0.80 b	2.01 cd	1.66 c	2.39 de	2.03 e
2.0	0.58 d	0.85 bc	1.90 cd	0.91 b	1.88 bc	0.80 b	2.05 c	1.74 bc	2.49 cd	2.15 d
4.0	1.10 ab	0.99 ab	2.15 cd	1.59 a	1.90 b	1.41 a	2.08 c	1.83 b	2.65 c	2.33 c
8.0	1.03 b	1.05 a	2.45 cd	1.34 a	1.89 bc	1.43 a	2.20 b	1.88 b	3.01 b	2.51 b
16.0	1.25 a	1.09 a	2.60 d	1.30 a	2.06 a	1.36 a	2.73 a	2.08 a	3.70 a	2.63 a
Δ mean	0.67	0.45	1	0.65	0.5	0.27	0.95	0.57	1.47	0.8

Different lowercase letters within a column are significantly different (P<0.05), according to the multiple range Duncan test.
Δ mean: difference between the smallest and largest value

Regarding the effect of the treatments, Ca^{+2} had a proportional increase in the interchangeable phase in all the soils evaluated regarding the control treatment (Table 4). This effect agrees with the reports of Alva *et al.* (1988), Van Raij (1988), Espinosa and Lobo (1999), Salas *et al.* (2002), and Elrashidi *et al.* (2010), in Ultisols, Oxisols and Mollisols, using a product similar to AG. Additionally, the same trend was seen even in the Andisols, Entisol and Inceptisol evaluated since all soils showed a significant difference in the increase in exchangeable Ca^{+2} with respect to the control.

In general, the soil Ca^{2+} saturation significantly (P<0.05) increased with the increase of the AG doses, particularly with the highest dose. However, the magnitude of the increase varied according to the soil type (Figure 3). In the Anserma and Libano soils, there were significant differences only with the highest AG dose used.

Table 4. Soil exchangeable Ca^{2+} after two weeks of incubation with AG. (Vertical comparison)

AG dose (g kg^{-1})	Soil exchangeable Ca^{+2} ($cmol_c$ kg^{-1})									
	La Tebaida	Anserma	Frontino	Líbano	Palestina	Palermo	Manizales	Zapatoca	Caucasia	Carimagua
0.0	2.09 d	8.69 d	1.25 f	4.41 e	3.83 f	6.28 d	3.19 g	1.88 f	0.09 g	0.12 g
0.25	2.25 d	8.34 d	1.69 f	4.80 e	4.14 f	7.15 d	3.31 fg	1.81 f	1.83 f	1.80 f
0.5	2.62 d	8.68 d	2.15 ef	5.72 de	4.55 f	6.46 cd	3.64 f	2.64 f	2.72 e	3.47 e
1.0	2.86 d	8.28 d	3.13 de	6.48 cd	6.70 e	7.18 cd	5.17 e	6.89 e	4.36 d	3.82 e
2.0	5.63 c	11.34 c	3.56 d	6.41 cd	8.27 d	8.17 c	7.23 d	8.59 d	6.52 c	5.79 d
4.0	5.68 c	13.20 c	6.48 c	8.12 b	9.54 c	9.85 b	9.65 c	12.11 c	6.44 c	7.98 c
8.0	10.39 b	15.96 b	11.16 b	7.46 bc	11.45 b	11.03 ab	10.61 b	14.59 b	10.05 b	10.10 b
16.0	15.18 a	33.15 a	18.78 a	17.89 a	13.59 a	12.33 a	13.93 a	35.71 a	25.70 a	17.60 a
Δ mean	13.09	24.87	17.53	13.48	9.76	6.05	10.74	33.9	25.61	17.48

Different lowercase letters within a column are significantly different (P<0.05), according to the multiple range Duncan test.
Δ mean: difference between the smallest and largest value

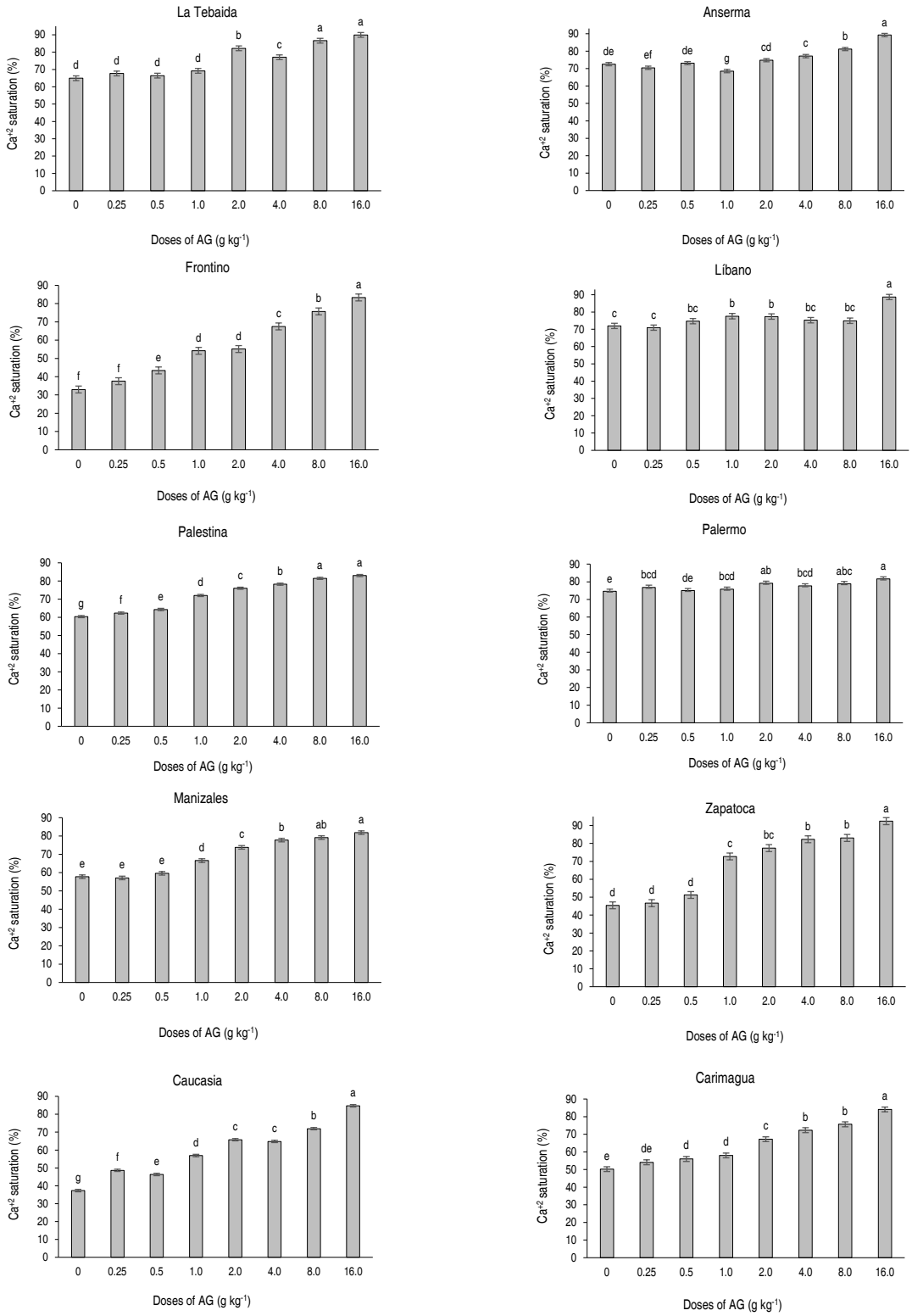


Figure 3. Soil Ca^{+2} saturation (%) after two weeks of incubation with AG. Columns with different lowercase letters mean significant differences (P<0.05), according to Duncan test.

There was a significant increase in the soil $S\text{-}SO_4^{-2}$ concentration as a result of the AG application. In many cases, even with the lower AG doses (0.25 g kg^{-1}), there was an increase in the value of soil $S\text{-}SO_4^{2-}$ concentration. The minimum Δ mean increase according to the unfertilized control was 13.72 mg kg^{-1} (Anserma soil) and the maximum was 28.28 mg kg^{-1} (Manizales soil) (Table 5). Clearly, it shows that the AG used is an efficient source to provide $S\text{-}SO_4^{-2}$ to soils and plants. Similar results were obtained by Salas *et al.* (2002) in Costa Rica and Alva *et al.* (1988) with doses comparable to those applied in this experiment (2 and 10 Mg ha^{-1}) in a Typic Hapludult. Besides, Elrashidi *et al.* (2010) found similar behavior in a Typic Argiudoll.

Table 5. Soil $S\text{-}SO_4^{-2}$ concentration after two weeks of incubation with AG. Means followed by different lowercase letters are significantly different (P<0.05), according to the multiple range Duncan test. Vertical comparisons.

AG dose (g kg^{-1})	Soil $S\text{-}SO_4^{-2}$ concentration (mg kg^{-1})									
	La Tebaida	Anserma	Frontino	Líbano	Palestina	Palermo	Manizales	Zapatoca	Caucasia	Carimagua
0.0	3.20 f	7.08 c	7.53 f	5.79 e	4.82 g	3.13 f	2.35 h	2.57 h	2.27 e	0.33 g
0.25	5.96 e	8.21 c	11.37 e	8.39 de	7.39 f	6.74 e	5.99 g	4.69 g	3.18 e	1.03 g
0.5	16.05 d	7.90 c	12.11 e	9.13 d	7.52 f	11.36 d	8.31 f	7.55 f	3.93 e	4.61 f
1.0	17.08 cd	14.38 b	14.93 d	13.82 c	10.97 e	14.29 c	17.03 e	10.35 e	5.76 d	6.48 e
2.0	18.17 c	18.94 a	18.86 c	16.41 c	17.22 d	15.81 c	23.48 d	13.44 d	12.51 c	9.56 d
4.0	22.62 b	19.47 a	19.25 c	19.67 b	19.10 c	18.22 b	25.49 c	17.20 c	22.21 b	14.87 c
8.0	24.83 a	20.80 a	22.84 b	19.48 b	21.31 b	27.82 a	28.04 b	21.66 b	23.46 b	23.23 b
16.0	26.30 a	18.92 a	27.16 a	25.31 a	26.40 a	27.83 a	30.63 a	30.37 a	29.28 a	26.23 a
Δ mean	23.09	13.72	19.63	19.51	21.57	24.70	28.28	27.80	27.00	25.90

Different lowercase letters within a column are significantly different (P<0.05), according to the multiple range Duncan test.
Δ mean: difference between the smallest and largest value

The addition of AG had significant effects on the soil exchangeable Mg^{2+} in La Tebaida, Líbano, Palestina, Manizales, and Caucasia. However, the magnitude of this effect varied among soils and doses. There was not a clear tendency to increase or decrease the level of this variable as the AG dose increase. Higher values of soil exchangeable Mg^{2+} were detected with different intermediate AG doses. It is interesting to note that even with the highest dose (16.0 g kg^{-1}), not significant decreases were observed, despite being applied in several soils with high sand contents (between 54 and 66%) and low Caption Exchange Capacity (CEC) (Table 6). The results

Table 6. Soil exchangeable Mg^{2+} after two weeks of incubation with AG. (Vertical comparison)

AG dose (g kg^{-1})	Soil exchangeable Mg^{2+} ($cmol_c$ kg^{-1})									
	La Tebaida	Anserma	Frontino	Líbano	Palestina	Palermo	Manizales	Zapatoca	Caucasia	Carimagua
0.0	0.42 ab	2.68 a	0.81 a	0.78 b	0.68 b	1.22 a	0.53 b	0.16 a	0.89 a	0.05 a
0.25	0.30 c	2.73 a	0.86 a	0.97 ab	0.75 a	1.27 a	0.56 b	0.14 a	0.81 c	0.04 a
0.5	0.41 ab	2.37 a	0.94 a	0.99 a	0.65 b	1.31 a	0.55 b	0.17 a	0.88 ab	0.06 a
1.0	0.34 bc	2.79 a	0.86 a	0.83 ab	0.68 b	1.46 a	0.57 ab	0.16 a	0.88 ab	0.06 a
2.0	0.50 a	2.71 a	0.86 a	0.80 ab	0.68 b	1.30 a	0.50 b	0.17 a	0.88 ab	0.05 a
4.0	0.46 a	2.62 a	0.85 a	0.93 ab	0.69 ab	1.32 a	0.67 a	0.17 a	0.83 bc	0.06 a
8.0	0.42 ab	2.62 a	0.85 a	0.93 ab	0.69 b	1.32 a	0.67 ab	0.17 a	0.83 ab	0.06 a
16.0	0.49 a	2.57 a	1.04 a	0.82 ab	0.66 b	1.35 a	0.58 ab	0.14 a	0.90 a	0.05 a
Δ mean	0.20	0.22	0.23	0.21	0.10	0.24	0.17	0.03	0.10	0.02

Different lowercase letters within a column are significantly different (P<0.05), according to the multiple range Duncan test.
Δ mean: difference between the smallest and largest value

differ from the data reported by other authors in soils susceptible to leaching, especially with a high content of sand and low CEC (Van Raij, 1988; Barceló *et al*., 1996; Ernani *et al*., 2006).

There were significant differences in the values of soil exchangeable K^+ associated with the AG treatments except for Caucasia and Carimagua soils. With the exception of the Manizales soil, the other treatments showed increases concerning the control treatment (Table 7). Similar results were reported by Espinosa and Lobo (1999) in the soils of Venezuela, Salas *et al*. (2002) in Ultisols and Andisols of Costa Rica, and Van Raij (1988) in Oxisols of Brazil. Thus, the maximal values were detected either with the unfertilized control or with intermediate and/or the highest AG dose. The magnitude of the changes was small, as shown in Table 7.

Table 7. Soil exchangeable K^+ after two weeks of incubation with AG.

AG dose ($g\ kg^{-1}$)	Soil exchangeable K^+ ($cmol_c\ kg^{-1}$)									
	La Tebaida	Anserma	Frontino	Líbano	Palestina	Palermo	Manizales	Zapatoca	Caucasia	Carimagua
0.0	0.13 ab	0.28 ab	0.09 c	0.14 c	0.05 ab	1.21 c	1.10 a	0.54 bc	0.77 ab	0.03 ab
0.25	0.12 cd	0.26 bc	0.11 b	0.16 ab	0.05 ab	1.95 a	0.91 ab	0.51 c	0.80 ab	0.04 a
0.5	0.13 bc	0.27 b	0.11 b	0.16 ab	0.05 ab	1.99 a	0.60 c	0.72 a	0.82 ab	0.03 a
1.0	0.12 d	0.29 ab	0.11 b	0.16 ab	0.03 b	1.47 bc	0.80 bc	0.79 c	0.84 a	0.02 b
2.0	0.14 abc	0.24 c	0.11 b	0.17 a	0.05 a	1.77 ab	0.84 bc	0.59 abc	0.81 ab	0.04 a
4.0	0.14 ab	0.28 ab	0.12 ab	0.16 b	0.05 a	1.86 ab	0.85 ab	0.61 abc	0.79 ab	0.03 ab
8.0	0.14 a	0.25 c	0.13 a	0.16 bc	0.04 ab	1.69 ab	0.90 ab	0.91 abc	0.72 b	0.03 ab
16.0	0.11 a	0.30 a	0.12 ab	0.16 ab	0.05 ab	1.74 ab	1.00 ab	0.70 ab	0.72 b	0.03 a
Δ mean	0.04	0.06	0.03	0.03	0.02	0.79	0.50	0.40	0.12	0.01

Different lowercase letters within a column are significantly different ($P \leq 0.05$), according to the multiple range Duncan test.
Δ mean: difference between the smallest and largest value

Although there were significant differences in the values of soil soluble P concentration associated to the AG treatments, there was not a tendency to increase or decrease it while the AG dose increase (Table 8). Similar results were obtained by Salas *et al.* (2002) in Ultisols and Andisols of Costa Rica.

Table 8. Soil soluble P concentration after two weeks of incubation with AG. Means followed by different lowercase letters are significantly different (P<0.05), according to the multiple range Duncan test. Vertical comparisons.

AG Dose ($g\ kg^{-1}$)	Soil soluble P concentration ($mg\ L^{-1}$)									
	La Tebaida	Anserma	Frontino	Líbano	Palestina	Palermo	Manizales	Zapatoca	Caucasia	Carimagua
0.0	0.021 ab	0.013 c	0.014 a	0.018 a	0.019 a	0.020 a	0.069 a	0.079 a	0.239 a	0.269 a
0.25	0.018 b	0.015 bc	0.014 a	0.019 bc	0.017 a	0.014 a	0.064 ab	0.018 a	0.028 b	0.022 b
0.5	0.016 b	0.016 ab	0.014 a	0.014 ab	0.016 a	0.016 a	0.063 ab	0.020 a	0.027 b	0.022 b
1.0	0.013 c	0.016 abc	0.013 a	0.016 bc	0.014 c	0.011 b	0.061 ab	0.024 a	0.028 b	0.020 b
2.0	0.013 c	0.017 ab	0.010 a	0.020 bc	0.014 ab	0.015 b	0.048 c	0.021 a	0.022 b	0.081 b
4.0	0.017 b	0.014 bc	0.014 a	0.017 bc	0.015 a	0.014ab	0.058 ab	0.017 a	0.022 b	0.022 b
8.0	0.012 b	0.010 bc	0.011 a	0.009 ab	0.009 abc	0.011 b	0.039 b	0.013 a	0.016 b	0.036 b
16.0	0.021 a	0.018 a	0.014 a	0.017 c	0.013 ab	0.008 b	0.044 c	0.060 a	0.021 b	0.024 b
Δ mean	0.009	0.008	0.004	0.011	0.011	0.012	0.030	0.066	0.223	0.249

Different lowercase letters within a column are significantly different ($P<0.05$), according to the multiple range Duncan test.
Δ mean: difference between the smallest and largest value

CONCLUSIONS

Higher doses of gypsum can cause a small decrease in pH and increase soil exchangeable Al^{+3}. The addition of AG can be useful to provide exchangeable Ca^{2+} and $S\text{-}SO_4^{2-}$ to agricultural soils and thus increase soil Ca^{2+} saturation, and parallelly, reduce soil Al^{3+} saturation. In most soils, these changes were detectable even when the AG doses were low (0.25 to 1.0 g kg^{-1}). The effects tend to be higher when increasing AG doses, but the magnitude of these varies among soils. In general, there were not significant trends with the AG addition and the values of soil exchangeable Mg^{+2} and K^{+} and soluble P. The results indicate that incubation is an appropriate and relatively fast method to detect changes in some soil fertility parameters.

REFERENCES

Alva AK, Summer ME and Noble AD. 1988. Alleviation of aluminum toxicity by phosphogypsum. Communications in Soil Science and Plant Analysis 19(4): 385–403. doi: 10.1080/00103628809367947

Baldión JV y Guzman O. 2008. Caracterización del clima y de la disponibilidad hídrica en el ecotopo 206A. Departamentos de Caldas y Risaralda. FNC-Cenicafé, Chinchiná. pp. 3-7.

Baldión J y Guzman O. 2009. Caracterización del clima y de la disponibilidad hídrica en el ecotopo 107B. Departamentos de Caldas y Risaralda. FNC-Cenicafé, Chinchiná. pp. 3-5.

Barceló J, Poschenrieder Ch, Vázquez MD and Gunsé B. 1996. Aluminium phytotoxicity: A challenge for plant scientists. Fertilizer Research 43: 217-223. doi: 10.1007/BF00747705

Brown JR. 1987. Soil testing: sampling, correlation, calibration, and interpretation (Vol. 21). Soil Science Society of America, Inc., Madison. pp. 1-14.

Castro HE y Gómez MI. 2013. Fertilidad de suelos y fertilizantes. pp. 217-280. En: Burbano Orjuela H y Silva Mojica F (eds.). Ciencia del Suelo: Principios Básicos. Segunda edición. Sociedad Colombiana de la Ciencia del Suelo, Bogotá. 594 p.

Elrashidi MA, West LT, Seybold CA, Benham EC, Schoeneberger PJ and Ferguson R. 2010. Effects of gypsum addition on solubility of nutrients in soil amended with peat. Soil Science 175(4): 162-172. doi: 10.1097/SS.0b013e3181dd51d0

Ernani PR, Miquelluti DJ, Fontoura SMV, Kaminski J and Almeida JA. 2006. Downward movement of soil cations in highly weathered soils caused by addition of gypsum. Communications in Soil Science and Plant Analysis 37(3-4): 571–586. doi: 10.1080/00103620500449443

Espinosa JG y Lobo D. 1999. Efectos de la aplicación de fosfoyeso sobre algunas propiedades de un suelo franco-arenoso. Venesuelos 7(1-2): 7-11.

Favarin JL, Moscardini DB, De Souza LT e Baptistella JLC. 2018. Camhinos para aumentar a produtividade do café arábica. Informações Agronômicas No. 164. 13 p.

Fisher M. 2011. Amending soils with gypsum. Crops and soils magazine. American Society of Agronomy, Madison. pp. 4-9.

Gómez L, Caballero A y Baldión JV. 1991. Ecotopos cafeteros de Colombia. Santafé de Bogotá, Federación Nacional de Cafeteros-FNC, Santa Fe de Bogotá. 131 p.

González H. 2013. Identificación de las principales Unidades de Suelos de la zona cafetera. pp. 269-283. En*:* Manual del Cafetero Colombiano. Tomo 1. FNC-Cenicafé, Santafé de Bogotá. 320 p.

Havlin JL, Tisdale SL, Nelson WL and Beaton JD. 1999. Soil fertility and fertilizers. An introduction to nutrient management. Sixth edition. Prentice-Hall Inc, New Jersey. 507 p .

IGAC - Instituto Geográfico Agustín Codazzi. 1995. Suelos de Colombia. IGAC, Bogotá. Colombia. 632 p.

IGAC - Instituto Geográfico Agustín Codazzi. 2014. Manejo de suelos colombianos. Imprenta Nacional de Colombia S.A., Bogotá. 323 p.

IGAC - Instituto Geográfico Agustín Codazzi. 2015. Suelos y tierras de Colombia. Tomo I. Imprenta Nacional de Colombia S.A, Bogotá. 854 p.

Kalinitchenko V and Nosov V. 2019. Phosphogypsum: P Fertilizer by-product and soil amendment. Better Crops 103(1): 50-53.

Madero ME. 2013. Principios elementales de génesis y clasificación de suelos. pp 5-71. En: Burbano Orjuela H y Silva Mojica F (eds.). Ciencia del Suelo: Principios Básicos. Segunda edición. Sociedad Colombiana de la Ciencia del Suelo, Bogotá. 594 p.

Osorio NW. 2018. Manejo de Nutrientes en Suelos del Trópico. Segunda Edición. L. Vieco SAS, Medellín. 411 p.

Osorno H. 2012. Mitos y realidades de las cales y enmiendas en Colombia (Tesis de Maestría). Universidad Nacional de Colombia. Medellín, Colombia. 70 p.

Prochnow LI, Caires E and Rodrigues C. 2016. Phosphogypsum use to reduce subsoil acidity: the Brazilian experience. Better Crops with plant foods 100(2): 13-15.

Ramos BZ, Toledo JPV, Lima JMD. Serafim ME, Bastos ARR, Guimarães PTG e Coscione AR. 2013. Doses de gesso em cafeeiro: influência nos teores de cálcio, magnésio, potássio e pH na solução de um Latossolo Vermelho distrófico. Revista Brasileira de Ciência do Solo 37(4): 1018-1026. doi: 10.1590/S0100-06832013000400019

Sadeghian KS y Lynce SLA. 2014. Variabilidad del suelo en lotes cafeteros - consideraciones para el muestreo. Avance Técnico Cenicafé No. 446. FNC-Cenicafé, Chinchiná. 4 p.

Salas RE, Smyth TJ, Alpizar D, Boniche J, Alvarado A y Rivera A. 2002. Corrección de la acidez del suelo con Ca y Mg y su efecto en el desarrollo del sistema radical del palmito en la etapa de previvero. Agronomía Costarricense 26(2): 87-94.

Saadaoui E, Ghazel N, Ben Romdhane C and Massoudi N. 2017. Phosphogypsum: potential uses and problems–a review. International Journal of Environmental Studies 74(4): 558-567. doi: 10.1080/00207233.2017.1330582

Takahashi T, Ikeda Y, Nakamura H and Nanzyo M. 2006. Efficiency of gypsum application to acid Andosols estimated using aluminum release rates and plant root growth. Soil Science and Plant Nutrition 52(5): 584-592. doi: 10.1111/j.1747-0765.2006.00071.x

Uchida R and Hue NV. 2000. Soil Acidity and Liming. pp. 101-111. In: Silva JA and Uchida R (eds.). Plant Nutrient Management in Hawaii's Soils, Approaches for Tropical and Subtropical Agriculture. College of Tropical Agriculture and Human Resources, University of Hawaii, Manoa. 158 p.

USDA. 2010. Keys to soil taxonomy. Eleventh edition,

Washington. 365 p.

Van Raij B. 1988. Gesso agricola na melhoria do ambiente radicular no subsolo. Associação Nacional para Difusao de Adubos e Corretivos Agrícolas, São Paulo. 88 p.

Van Raij B. 2008. Gesso na agricultura. Instituto Agronômico Campinas (SP), Brasil. 233 p.

Von Uexkull HR. 1986. Efficient fertilizer use in acid upland soils of the humid tropics. Food and Agriculture Organization of the United Nations-FAO, Roma. 59 p.

Zapata R. 2014. Los procesos químicos del suelo Primera Edición. Universidad nacional de Colombia. Facultad de ciencias. Escuela de Geo ciencias, Medellín. pp.167-177.

ÍNDICE DE AUTORES

POLÍTICA EDITORIAL

REVISTA FACULTAD NACIONAL DE AGRONOMÍA MEDELLÍN

La Revista Facultad Nacional de Agronomía Medellín (RFNA), es una publicación de la Facultad de Ciencias Agrarias de la Universidad Nacional de Colombia - Sede Medellín. Esta orientada a profesores, investigadores, estudiantes, extensionistas y a todos aquellos profesionales que crean conocimiento y articulan la ciencia y la tecnología para hacer más productivo el campo a nivel empresarial y de economía campesina.

La Revista recibe y publica, sin ningún costo, artículos en idioma inglés de investigación, revisión, reseñas, cartas al editor y editoriales.

La periodicidad de la Revista es cuatrimestral, con circulación nacional e internacional y tiene como objetivo divulgar artículos escritos en inglés, originales, inéditos y arbitrados (peer review) de carácter científico que respondan a preguntas específicas y que proporcionen soporte y pruebas a una hipótesis, en aspectos relacionados con las Ciencias Agronómicas, Zootecnia, Ciencias Forestales e Ingeniería Agrícola y de Alimentos y otras afines que contribuyan a la solución de los limitantes del agro en el trópico.

Teniendo en cuenta los criterios considerados por Colciencias, la revista acoge documentos de las siguientes tipologías:

Artículos de investigación científica y tecnológica: Documento que presenta, de manera detallada, los resultados originales de proyectos terminados de investigación. La estructura generalmente utilizada contiene cuatro partes fundamentales: Introducción, metodología (materiales y métodos), resultados y discusión, y conclusiones. La extensión máxima debe ser de 5200 palabras, excluyendo figuras, tablas, referencias. El mínimo de referencias bibliográficas sugerido es de 10 y el máximo de 30. Este tipo de artículos es arbitrado e indexado.

Artículos de revisión: Documentos producto de una investigación terminada donde se analizan, sistematizan e integran los resultados de investigaciones publicadas o no publicadas, sobre un campo en ciencia o tecnología, con el fin de dar cuenta de los avances y las tendencias de desarrollo. Se caracteriza por presentar una cuidadosa revisión bibliográfica de por lo menos 50 referencias. La extensión máxima debe ser de 6000 palabras, excluyendo figuras, tablas, referencias. Este tipo de artículos es arbitrado e indexado.

Artículos cortos: Documento breve que presenta resultados originales preliminares o parciales de una investigación científica o tecnológica, que por lo general requieren de una pronta difusión. Para todos los casos el 60% de las citas debe provenir de artículos publicados en los últimos diez años.

Los artículos deben ser presentados de acuerdo a los lineamientos establecidos en las “Instrucciones a los Autores”; quienes incumplan las normas básicas no iniciarán el proceso editorial. Se debe diligenciar el formato “Autorización para Publicación de Obras y Cesión de Derechos Patrimoniales”, el cual será suministrado por la Revista. Dicho documento es explícito en mencionar que todos los autores están informados y de acuerdo con someter el artículo a consideración de la Revista, que no hay conflictos de interés entre ellos y expresa que el contenido del manuscrito no ha sido ni será enviado para su publicación a otra Revista.

El Comité Editorial, apoyado por un equipo de editores asociados, evaluará el mérito científico del documento y luego lo someterá a evaluación bajo la modalidad doble ciego -es decir que se guarda estricto anonimato en la revisión- por dos árbitros especializados en el tema, preferiblemente uno nacional y otro internacional, quienes entregarán su dictamen en el formato establecido por la Revista. El Comité Editorial se reserva el derecho de aceptar o no las colaboraciones. El dictamen luego del proceso de revisión puede ser: aceptado para publicación sin ninguna o pocas modificaciones; aceptado para publicación con cambios mayores de acuerdo a las observaciones de los evaluadores; reconsiderado para publicación si se modifica sustancialmente, en este caso, será catalogado como material nuevo; rechazado para publicación. Si los artículos son aceptados, estos serán devueltos a los autores para su corrección y remitidos de nuevo al Director de la Revista en los siguientes 30 días calendario.

La impresión de gráficos, figuras o fotografías en color es opcional y tiene un costo adicional por página necesaria de cien mil pesos colombianos ($100.000). La redacción de la Revista se reserva el derecho de realizar modificaciones de forma en el texto del artículo (títulos, resúmenes/abstracts, tablas y figuras); siempre que sea posible, se consultará a los autores sobre los cambios introducidos.

El autor(es) se compromete(n) a ceder los derechos de impresión y reimpresión del material publicado a la Revista Facultad Nacional de Agronomía y cualquier cita a los artículos editados en la Revista se deberá hacer si se adiciona el crédito respectivo. En caso de duplicación del contenido de la Revista o su publicación parcial o total en otro idioma, se deberá contar con el permiso escrito del Director.

La Revista admite comentarios y opiniones que discrepen de los términos expresados en el material publicado, acepta retractaciones argumentadas de los autores y corregirá los errores tipográficos y de otra índole que se puedan haber cometido al publicar un artículo. La Facultad de Ciencias Agrarias y la Revista no se responsabilizan o solidarizan, necesariamente, con los conceptos emitidos en los artículos publicados, cuya responsabilidad será en su totalidad del autor o los autores.

Para mayor información, correspondencia, suscripciones y canje, dirigirse a la Universidad Nacional de Colombia - Sede Medellín, Facultad de Ciencias Agrarias, Revista Facultad Nacional de Agronomía Medellín. Apartado Aéreo 568, Medellín, Colombia. Teléfono: (4) 430 9006; Fax: (4) 230 0420; correo electrónico: rfnagron_med@unal.edu.co La Revista puede consultarse en su versión electrónica en http://www.revistas.unal.edu.co/index.php/refame

INSTRUCCIONES A LOS AUTORES

Lineamientos generales

Los artículos deben ser enviados a través del Open Journal System en el Portal de Revistas de la Universidad Nacional de Colombia http://www revistas.unal.edu.co/. Sólo serán considerados artículos escritos en inglés. Adjunto se deben remitir los siguientes cuatro formatos: (1) Lista de verificación de criterios editoriales para la presentación de manuscritos; (2) Autorización de publicación de manuscritos en la Revista Facultad Nacional de Agronomía Medellín, en la cual se acepta la no postulación simultánea del artículo a otras revistas u órganos editoriales y se ceden los derechos a la Revista para su difusión, este debe ser firmado por todos los autores del manuscrito; (3) Datos personales de cada autor; (4) Sugerencia de posibles pares evaluadores. Las formas de publicación son: artículos de investigación científica y tecnológica, artículos de revisión y artículos cortos. Los artículos pueden ser elaborados por profesores y/o investigadores de la Universidad Nacional de Colombia, o cualquier otra institución afín, nacional o internacional, en los temas Agropecuarios, Forestales y de Ingeniería Agrícola y de Alimentos. El manuscrito no debe exceder 5200 palabras para artículos de investigación y 6000 para artículos de revisión. Las hojas deben ser tamaño carta, escritas a interlineado doble, numeración de línea continua, letra o fuente Times New Roman o Verdana, tamaño 12 puntos, márgenes de 3 cm en la parte superior, 2 cm en la inferior y 2,5 cm en las márgenes laterales derecha e izquierda. Las tablas y figuras (es decir, los gráficos, dibujos, esquemas, diagramas de flujo, fotografías y mapas) se deben mostrar incorporadas en el texto y con numeración consecutiva (Tabla 1... Tabla n; Figura 1... Figura n, etc.). Los textos y tablas se deben presentar en el procesador de palabras MS-Word®;las tablas y los diagramas de frecuencia (barras y tortas) originales se deben suministrar en el archivo del documento y también en su original de MS-Excel®; otras figuras, como fotografías sobre papel y dibujos, se pueden enviar en original o escaneadas y ser remitidas en el formato digital de compresión JPG (o JPEG) preferiblemente con una resolución de 600 x 600 dpi (mínimo 300 dpi); es deseable que las fotografías originales sean enviadas como diapositivas. Como norma general, las tablas y figuras sólo se aceptan en blanco y negro; excepcionalmente se incluirán en color cuando sea estrictamente necesario y a juicio del Comité Editorial.

Unidades, abreviaturas y estilo

Se debe utilizar el Sistema Internacional de Unidades (SIU), y aquellas unidades específicas de mayor uso por parte de la comunidad científica. Las unidades combinadas deben usar la forma exponencial. Ejemplo: kg ha^{-1}. El significado de las abreviaturas debe citarse por extenso cuando se mencionan por primera vez en el manuscrito. El estilo de escritura debe ser absolutamente impersonal, en tiempo gramatical pasado para la introducción, los procedimientos y los resultados y presente para la discusión, evitando la conjugación de verbos en primera o tercera persona del singular o el plural.

Los números del uno al nueve se escriben en palabras, excepto cuando incluyen unidades de medida o se mencionan varios números. Ejemplo: “ocho tratamientos”, “3, 7 y 9 lecturas”, “15 kg”. Use cero antes del punto decimal. Para separar números en intervalos de uno o más años, use la letra “a”, y guión para temporadas de crecimiento. Ejemplo: Periodo 2002 a 2005; temporadas de crecimiento 1999-2000, 2000-2001.

Título y autores

El título del artículo no debe incluir abreviaturas y es obligatoria su respectiva traducción al idioma español. En lo posible, el título no debe exceder de 15 palabras y debe reflejar con precisión el contenido del documento. Cuando contenga nombres científicos de especies vegetales o animales, éstos se deben escribir con letra cursiva (itálica) en minúsculas, sólo con mayúsculas la primera letra del género y del clasificador. Debajo del título en inglés se escribe el nombre(s) y apellido(s) de los autores, sin sus respectivos títulos académicos, ni cargos laborales, en una línea horizontal y de acuerdo con su contribución en la investigación y/o preparación del artículo.

Como nota al pie de la primera página, se escribe el título de pregrado, el cargo laboral de los autores, el nombre y la ciudad de ubicación de la entidad a la cual prestan sus servicios o del patrocinador para la realización del trabajo y su respectiva dirección de correo electrónico, indicando el autor de correspondencia. Además, se debe adjuntar un resumen de la hoja de vida de los autores, donde se mencionen los artículos publicados en otras revistas.

Resumen, abstract y palabras claves

El resumen no debe exceder de 250 palabras escritas en un único párrafo. Se debe escribir en inglés y español. Debe contener en forma breve la justificación, los objetivos, los métodos utilizados, los resultados obtenidos más relevantes y las conclusiones. Es obligatorio acompañar el resumen con un máximo de seis palabras clave distintas a las utilizadas en el título. Se aceptan como palabras clave no sólo las palabras simples, sino también términos compuestos hasta de tres palabras. Deben ir escritas en minúsculas y separadas por comas.

Introducción

Puede tener o no título. Define el problema e informa sobre el estado del arte respecto al tema principal del artículo; además, señala las razones que justifican la investigación y plantea los objetivos de la misma. Es obligatorio acompañar los nombres vulgares con el nombre(s) científico(s) y la abreviatura(s) del clasificador en la primera mención dentro del texto. No se deben mencionar marcas de productos, sino su nombre genérico o químico

Materiales y métodos

En este apartado se deben describir en forma clara, concisa y secuencial, los materiales (vegetales, animales, implementos agrícolas o de laboratorio) utilizados en el desarrollo del trabajo; además, se mencionan los aspectos relacionados con la ubicación, preparación y ejecución de los experimentos. Se debe indicar el diseño seleccionado, las variables registradas, las transformaciones hechas a los datos, los modelos estadísticos usados y el nivel de significancia empleado. Evitar detallar procedimientos previamente publicados.

Resultados y discusión

Son la parte central del artículo, deben estar respaldados por métodos y análisis estadísticos apropiados. Se deben presentar de manera lógica, objetiva y secuencial mediante textos, tablas y figuras; estos dos últimos apoyos deben ser fáciles de leer, autoexplicativos y estar siempre citados en el texto. Las tablas se deben elaborar con pocas columnas y renglones. Se debe tener la precaución de incluir el nivel de significancia estadística representado por letras minúsculas del comienzo del alfabeto (a, b, c, d,...), un asterisco simple (*) para $P<0,05$, doble asterisco (**) para $P<0,01$ o triple asterisco (***) para $P<0,001$. Las investigaciones que no siguen un diseño estadístico, deben mostrar la información de manera descriptiva. Use subíndices para modificaciones, reserve superíndices para potencias o notas al pie en tablas y figuras.

La discusión: Se refiere al análisis e interpretación objetiva de los resultados, confrontándolos con los obtenidos en otras investigaciones, o con los hechos o teorías conocidos sobre el tema. Explica los resultados en particular cuando difieren de la hipótesis planteada. Destaca la aplicación práctica o teórica de los resultados obtenidos y

las limitaciones encontradas. Resalta la contribución que se hace a una determinada área del conocimiento y el aporte a la solución del problema que justifica la investigación. Finalmente, proporciona elementos que permitan proponer recomendaciones o lanzar nuevas hipótesis. No se deben hacer afirmaciones que van más allá de lo que los resultados pueden apoyar.

Conclusiones

Son las afirmaciones originadas a partir de los resultados obtenidos, deben ser coherentes con los objetivos planteados y la metodología empleada; además, expresar el aporte al conocimiento en el área temática estudiada y proponer directrices para nuevas investigaciones.

Agradecimientos

Si se considera necesario, se incluyen los agradecimientos o reconocimientos a personas, instituciones, fondos y becas de investigación, que hicieron contribuciones importantes en la concepción, financiación o realización de la investigación.

Formato de citación en el texto

- Se registra la fuente entre paréntesis, el cual debe incluir el apellido del autor y año, con coma entre autor y año. Ejemplo: (Pérez, 1995).

- Si hay más de una fecha se separarán con comas: Ejemplo: (Pérez, 1995, 1998, 2001)

- Si hay dos autores se citarán separados por la conjunción and. Ejemplo: (Gil and Ortega, 1993)

- Si hay varios trabajos de un autor publicados en un mismo año, se citarán con una letra en secuencia alfabética de los títulos, adosada al año. Ejemplo: (Gómez, 2000a, 2000b, 2000c)

- En el caso de citas con tres o más autores, es necesario mencionar en el texto el apellido del primero y reemplazar los demás por la expresión latina abreviada *et al.* (en cursiva) que significa y otros; en la referencia se deben poner los apellidos e iniciales de todos los autores. Ejemplo: (García *et al*., 2004).

- Cuando se hace referencia al autor dentro del texto, sólo se encierra el año entre paréntesis y se omite la coma que separa al autor del año. Ejemplo: (1) De acuerdo con Castañeda (2000), …; (2) Concorde con los resultados de Poveda *et al.* (2018) …

- Cuando es una cita de una cita se ponen la información de los autores citados y los autores citantes. Ejemplo: Magalhaes *et al.* (1979) expone que … (as cited in Gómez, 2004).

- Organizaciones se citan por sus siglas, en caso de no tener se cita con su nombre completo. Ejemplo: (1) (FAO, 2015), (2) (Ministerio de Agricultura y Ganadería, 2019)

Referencias

Sólo se listan las referencias bibliográficas mencionadas en el texto. No se aceptan notas de clase o artículos en preparación, o cualquier otra publicación de circulación limitada.

Las referencias bibliográficas se deben ordenar alfabéticamente por el apellido del primer autor, sin numeración y sin sangría. Para citar varias publicaciones del mismo autor, se debe seguir el orden cronológico creciente; si son del mismo año, se debe seguir el orden alfabético de los títulos.

Las referencias deberán contener todos los datos que permitan su fácil localización. Las referencias se citan en el lenguaje de publicación.

En cada referencia para todos los autores cite primero el apellido, tener en cuenta que algunos autores hispanos citan sus dos apellidos, seguido de la inicial del nombre sin puntos, separando autores con coma y espacio.

Ejemplos:

Libros: Autor(es). Año. Título del libro. Edición. Casa editora, ciudad de su sede. Páginas consultadas (pp. #-#) o páginas totales (# p.). Ejemplo: Robinson A, Morrison J, Muehrcke P, Kimerling AJ and Guptill S. 1995. Elements of Cartography. Sixth edition. John Wiley and Sons, Inc., New York. 674 p.

García Rodríguez JL, Giménez Suarez MC, Ortega Pérez E, Martín Ramos B, Calderón Guerrero C. 2014. Operaciones auxiliares en repoblaciones e infraestructuras forestales. Ediciones Paraninfo SA, Madrid. 208 p.

Capítulos de libros: Autor(es). Año. Título del capítulo. Páginas consultadas (pp. # - #). En: Apellidos e iniciales de los compiladores o editores (eds.). Título del libro. Edición. Casa editora, ciudad de su sede. Páginas totales (# p.). Ejemplo: Bernal H. 1996. Capítulo 6: Evapotranspiración. pp. 112-125. En: Agrios G. (ed.). Fitopatología. Segunda edición. Editorial Limusa, México D.F. 400 p.

Bertoft E and Blennow A. 2016. Chapter 3 - Structure of potato starch. pp 57-73. In: Singh J and Kaur L. (eds.). Advances in potato chemistry and technology. Second edition. Academic Press, London. 752 p.

Artículos de revistas: Autor(es). Año. Título del artículo. Nombre completo de la revista volumen(número de fascículo): página inicial–página final. doi. Ejemplo: García S, Clinton W, Arreaza L and Thibaud R. 2004. Inhibitory effect of flowering and early fruit growth on leaf photosynthesis in mango. Tree Physiology 24(3): 387-399. doi: 10.1093/treephys/24.4.387

Ponencias en memorias de congresos, seminarios, simposios: García M. 1998. La ingeniería geotécnica y la protección del medio ambiente. pp. 65-94. En: Memorias IX Congreso Colombiano de la Ciencia del Suelo. Sociedad Colombiana de la Ciencia del Suelo, Bogotá.

High R. 2015. Plotting LSMEANS and Differences in Generalized Linear Models with GTL. In: 2015 Midwest SAS Users Group Conference Proceedings. Midwest SAS Users Group, Omaha. 9 p.

Tesis, trabajos de grado. Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín, Colombia. 78 p.

Adam M. 1992. The Impact of the Common Agricultural Policy on Agriculture in Greece (Master's thesis). Cambridge University. Cambridge, United Kingdom. 80 p.

Cita de cita, sólo se referencia la fuente consultada. Ejemplo: Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín, Colombia.

Suplemento de revista: Silva AM y Carrillo NN. 2004. El manglar de piruja, Golfito, Costa Rica: un modelo para su manejo. Revista de Biología Tropical 52 Suppl. 2: 195-201.

Citas de internet: Autor(es). Año. Título del artículo. En: Nombre(s) de la publicación electrónica, de la página web, portal o página y su URL, páginas consultadas (pp. # - #) o páginas totales (# p.); fecha de consulta. Ejemplo: Arafat Y. 1996. Siembra de olivos en el desierto palestino. In: Agricultura Tropical, http://agrotropical.edunet.es. 25 p. consulta: noviembre 2003.

Patentes: Autor(es). Año. Título. País de la patente y número. Fuente. Ejemplo: Glenn RW. 1996. Liquid personal cleansing compositions which contain soluble oils and soluble synthetic surfactants. U.S. Patent No. 6194364. Retrieved from: https://patents.google.com/patent/US6194364B1/en

PUBLISHING POLICY

REVISTA FACULTAD NACIONAL DE AGRONOMÍA MEDELLÍN

The Journal *Revista Facultad Nacional de Agronomía Medellín* (RFNA) is published by the Faculty of Agricultural Sciences of Universidad Nacional de Colombia – Medellín. It is aimed at professors, researchers and students in agronomy, animal, and forestry sciences, food and agricultural engineering, agricultural advisers and at all those professionals who create knowledge and articulate science and technology to make the field more productive at business and rural economy levels.

The Journal receives and publishes, without any cost, research articles, reviews, revisions, letters to the editor and editorials written in the English language.

The Journal is a four-monthly publication at national and international level. Its aim is to publish original, unpublished, and peer-reviewed articles of a scientific nature which respond to specific questions and provide support and testing of a hypothesis, related to agronomy, animal husbandry, forestry engineering, food and agricultural engineering, and related areas that contribute to the solution of the agricultural constraints in the tropics.

Taking into account Colciencias (Administrative Department of Science, Technology and Innovation of Colombia) criteria, the journal welcomes papers of the following types:

Research papers in science and technology: A document presenting in detail the original results of completed research projects. The structure generally used contains four main parts: Introduction, methodology (materials and methods), results and discussion, and conclusions. The maximum extension must be 5200 words; excluding figures, tables, references. The maximum number of bibliographic references suggested is 30. This type of article is peer-reviewed and indexed.

Review articles: Documents resulted from a completed research systematizing, analyzing, and integrating the published or unpublished research findings, on a field of science or technology, in order to report the progress and development trends. It is characterized by a careful review of the literature of at least 50 references. The maximum length must be 6000 words; excluding figures, tables, references. This type of articles is arbitrated and indexed.

Short articles: short paper presenting original preliminary or partial results of a scientific or technological research, which usually require a quick diffusion. In all cases 60% of references must come from articles published in the last ten years.

Articles must be submitted in accordance with the guidelines set forth in “Instructions to Authors”; those who violate the rules will not initiate the basic editorial process. Shall be filled the form “Authorization for Release of Works and Economic Rights Assignment”, which will be provided by the Journal. This document is explicit in mentioning that all authors are informed and agree with article submitted for consideration to the Journal, that there is no conflict of interest between them, and also state that the manuscript has not been and will not be submitted for publication to another Journal.

The Editorial Board, supported by a team of associate editors, will evaluate the scientific merit of the paper and will then submit it for evaluation under double-blind method- that is to say, strict anonymity in the review is kept- by two arbitrators specialized in the area, preferably one national and one international, who will give their report on the format provided by the Journal. The Editorial Board reserves the right to accept collaborations. The report, after the review process, can be: accepted for publication with no or few modifications; accepted for publication with major changes according to the comments of the evaluators; reconsidered for publication if it is substantially modified - in this case, it will be deemed as new material; rejected for publication. If articles are accepted, they will be returned to authors for correction and sent again to the Director of the Journal within 30 calendar days.

Printing of graphs, figures or photographs in color is optional and have an additional cost per page needed of hundred thousand Colombian pesos ($ 100,000). The editorial staff of the Journal reserves the right to make editorial changes in the text of the article (titles, abstracts, tables and figures). Authors will be consulted on changes whenever it is possible.

The author or authors agree to assign the National Faculty of Agronomy Journal the printing and reprinting rights of the material published. Any reference to the articles published in the Journal should be done if proper credit is added. In case of duplication of the Journal content or its partial or total publication in another language, it must have written permission from the Director.

The Journals accepts comments and opinions differing from the terms expressed in the published manuscripts. It also accepts argued retractions from authors and will correct misprints and all kind of errors as may have been committed when publishing an article. The Faculty of Agronomy Sciences and the Journal are not responsible and do not necessarily sympathize with the concepts expressed in the articles, whose responsibility will be entirely the author´s or authors'.

For further information, correspondence, subscriptions and exchange, please contact: Universidad Nacional de Colombia - Sede Medellín, Facultad de Ciencias Agrarias, Revista Facultad Nacional de Agronomía. Apartado Aéreo 568, Medellín, Colombia. Tel: (4) 430 9006; Fax: (4) 230 0420; e-mail: rfnagron_med@unal.edu.co The Journal is available in its electronic version at http://www.revistas.unal.edu.co/index.php/refame

INSTRUCTIONS TO AUTHORS

General guidelines

Papers must be sent b through the Open Journal System in the Universidad Nacional de Colombia journals web side http://www.revistas.unal.edu.co/, Will be considered only papers written in English. The four following formats must be submitted with the manuscript: (1) Editorial Criteria Checklist for Paper Submission; (2) Paper Publishing Authorization for the Revista Facultad Nacional de Agronomía Medellín, which accepts no simultaneous nomination of the article to other journals or editorial bodies, and the rights are given to the Journal for its release by the signature of all the manuscript's authors; (3) Personal information of each author; (4) Suggestion of possible peer reviewers. Publishing forms are: scientific and technological research articles, review articles, reflection articles, and short articles. Articles can be developed by professors and/or researchers at the Universidad Nacional de Colombia, or other related national or international institution, on Agricultural, Forestry, Food and Agricultural Engineering matters. Article extension must not exceed 5,200 words for research articles and 6,000 words for reviews. The manuscript must be letter-size sheets, line spacing double, continuous line number 12 point Times New Roman or Verdana font, 3 cm margin at the upper, 2 cm in the lower, 2.5 cm on the left and right side margins. Tables and figures (i.e, graphics, drawings, diagrams, flowcharts, photographs and maps) should be shown on separate sheets and numbered consecutively (Table 1 ... Table n, Figure 1…Figure n, etc.). Texts and tables should be submitted in MS-Word® word processor, original tables and diagrams of frequency (bar charts and pie charts) must be supplied in manuscript file and in its original MS-Excel®; other figures, such as photographs on paper and drawings, can be sent in original or scanned and sent in digital format compression JPG (or JPEG), preferably with a resolution of 600 x 600 dpi (300 dpi at least); original photographs are suggested to be sent as slides. As a general rule, tables and figures are only accepted in black and white. Color figures will be exceptionally accepted when strictly necessary and under discretion of the Editorial Board.

Units, abbreviations and style

International System of Units (SI), and those specific units of greater use by the scientific community must be used. When required must be used the exponential form. Example: kg ha^{-1}. The meaning of abbreviations should be cited in full when first mentioned in the manuscript. The writing style should be totally impersonal. Introduction, procedures and results should be written in grammatical past tense. Discussion should be written in grammatical present tense, avoiding the conjugation of verbs in first or third person singular or plural.

The numbers from 1 to 9 are written in words, except when they include units of measure or several numbers are listed. Example: “eight treatments”, “3,7 and 9 readings”, “15 kg”. Use zero before the decimal point. To separate numbers in intervals of one to two years, use the letter “a” and hyphen for growing seasons. Example period 2002 to 2005, growing seasons 1999-2000, 2000-2001.

Title and authors

The article should not include abbreviations and its translation into English is required. As far as possible, the title should not exceed 15 words and must accurately reflect the paper content. When the article contains scientific names of plants or animals, they should be written in italics in lower case, only the first letter of gender and classifier should be capital. Under the title in English the author or authors' name (s) and surname (s) is /are written, without academic degrees or job positions, in a horizontal line according to the contribution to research and / or preparation of the article.

As a footnote on the first page, write the title of undergraduate, authors' job positions, the name and city location of the entity to which they serve, or the sponsors for the research work and their respective email address. In addition, a summarized authors' résumé including reference to the articles published in other magazines should be attached.

Abstract and key words

The abstract should not exceed 250 words written in a single paragraph. It must be written in English and Spanish. It should contain in brief the justification, aims, methods used, the most relevant results, and conclusions. It is required to accompany the abstract with a maximum of six key words, translated into English, different from those used in the title. Single words as well as compound terms of up to three words are accepted as key words. They must be written in lowercase, separated by commas.

Introduction

It may or not have a title. It defines the problem and reports on the state of the art on the main subject of the article, it also points out the reasons for the research and sets out its aims. It is required to accompany common names with the corresponding scientific name (s) name and abbreviation (s) of the classifier at the first mention in the text. Brands must not be mentioned but the generic or chemical name.

Materials and methods

In this section, materials (crops, livestock, agricultural or laboratory implements) used in the development of work should be clearly, concisely and sequentially described. Aspects related to the location, preparation and execution of experiments should also be mentioned. The selected design, the recorded variables, the changes made to data, the statistical models used and the significance level used should be indicated. Authors must avoid detailing procedures previously published.

Results

They are the central part of the article and must be supported by appropriate statistical methods and analysis. They should be presented in a logical, objective and sequential way through texts, tables and figures; the latter two supports should be easy to read, self- explanatory and always quoted in the text. The tables should be composed by few columns and rows. Care should be taken to include the statistical significance level represented by lowercase letters of the beginning of the alphabet (a, b, c, d,…), a single asterisk (*) for $P<0.05$, double asterisk (**) for $P<0.01$ or triple asterisk (***) for $P<0.001$. Researches that do not follow a statistical design should display the information in a descriptive way. Use subscripts to modifications, reserve superscripts for potencials or footnotes in tables and figures.

Discussion

It refers to the analysis and objective interpretation of results, comparing them with those obtained in other research, or with known facts or theories on the subject. It explains the results, especially when they differ from the stated hypothesis. It emphasizes the practical or theoretical application of the obtained results and constraints encountered. Discussion also highlights the contribution that is made to a particular area of knowledge and to the solution of the problem that justifies the research. Finally, it provides elements that allow making recommendations or launching new hypotheses. Statements that go beyond what the results may support should be avoided.

Conclusions

Conclusions are assertions arising from the obtained results. They should be consistent with the objectives stated and the methodology used. They should also express the contribution to knowledge in the studied subject area and propose guidelines for further researches.

Acknowledgements

If necessary, acknowledgements or recognitions to individuals, institutions, funds and research grants that made important contributions in the design, financing or carrying out of the research are included.

Citing in-text format

- Citations in the text should be in parenthesis and include author's surname and year, with comma in-between. Example: (Pérez, 1995).

- If more than one date, they are separated by commas: Example: (Pérez, 1995, 1998, 2001).

- If there are two authors, they will be separated by the conjunction and. Example: (Gil and Ortega, 1993)

- If there are several works of an author published in the same year, they will be cited with a letter in alphabetical sequence of titles, adjacent to year. Example: (Gómez, 2000a, 2000b, 2000c)

- For citations with three or more authors, it is necessary to mention in the text the surname of the first author and replace the others by the Latin expression *et al.* (in italics), which means and others. All authors should be mentioned in the reference. Example: (García *et al*., 2004)

- When the author is referenced within the text, only the year is enclosed in parentheses, and the comma that separates the author from the year is omitted. Example: (1) According to Castañeda (2000), ...; (2) In accordance with the results of Poveda *et al.* (2018), ...

- When an indirect source is cited, the information of the cited authors and the citing authors are placed. Example: (Magalhaes *et al.* (1979) state that ... (as cited in Gómez, 2004).

- Organizations are cited by their initials; in case they do not have their full name is used. Example: (1) (FAO, 2015), (2) (Ministerio de Agricultura y Ganadería, 2019)

References

Only bibliographical references cited in-text are listed in the references section. Lecture notes, articles in preparation, or any other publication with limited circulation are not accepted. Excessive self-citation should be avoided.

Bibliographic references are ordered alphabetically by first author's surname, without numbering and without indentation. To cite several publications of the same author, chronological increasing order must be followed. Alphabetical order of titles must be followed in case they are from the same year.

References should contain all the data allowing to its easy location. The titles of the papers, the surnames of the authors and the names of journals must be referenced and cited in their original language.

Examples:

For books: Author(s), Year. Book title, Edition, Publisher, Place of publication. Pages consulted (pp. #-#) or total pages.Example: Robinson A, Morrison J, Muehrcke P, Kimerling AJ and Guptill S. 1995. Elements of cartography. Sixth edition. John Wiley and Sons, Inc., New York. 674 p.

García Rodríguez JL, Giménez Suarez MC, Ortega Pérez E, Martín Ramos B, Calderón Guerrero C. 2014. Operaciones auxiliares en repoblaciones e infraestructuras forestales. Ediciones Paraninfo SA, Madrid. 208 p.

For book chapters: Author(s). year. Chapter title. pages consulted (pp. # - #). In: Surnames and names of the editors or publishers (eds.). book title. Edition. Publisher, place of publication. total pages (# p.). Example: Bertoft E and Blennow A. 2016. Chapter 3 - Structure of potato starch. pp 57-73. In: Singh J and Kaur L. (eds.). Advances in potato chemistry and technology. Second edition. Academic Press, London. 752 p.

Beral H. 1996. Capítulo 6: Evapotranspiración. pp. 112-125. En: Agrios G. (ed.). Fitopatología. Segunda edición. Editorial Limusa, México D.F. 400 p.

For journals: Author(s). year. Article title. journal full name volume(number): initial page-final page. Example: García S, Clinton W, Arreaza L and Thibaud R. 2004. Inhibitory effect of flowering and early fruit growth on leaf photosynthesis in mango. Tree Physiology 24(3): 387-399. doi: 10.1093/treephys/24.4.387

Presentations in Memoirs of Congresses, seminars and symposia: García M. 1998. La ingeniería geotécnica y la protección del medio ambiente. pp. 65-94. En: Memorias IX Congreso Colombiano de la Ciencia del Suelo. Sociedad Colombiana de la Ciencia del Suelo, Bogotá.

High R. 2015. Plotting LSMEANS and Differences in Generalized Linear Models with GTL. In: 2015 Midwest SAS Users Group Conference Proceedings. Midwest SAS Users Group, Omaha. 9 p.

Theses and dissertations: Adam M. 1992. The impact of the common agricultural policy on agriculture in Greece (Doctoral dissertation). Cambridge University. Cambridge, United Kingdom. 80 p.

Gómez C. 2004. Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín. Colombia. 78 p.

Citation of a citation, list the secondary source in your reference list: Example: Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín, Colombia. 78 p.

Journal Supplement: Silva AM y Carrillo NN. 2004. El manglar de piruja, Golfito, Costa Rica: un modelo para su manejo. Journal of Tropical Biology 52 Suppl. 2: 195-201.

For internet citations: Author (s), year. Article. In: electronic publishing Name (s), the web page, portal or page name and its URL, pages consulted (pp. # - #) or total pages (# p.), date of consultation. Example: Arafat Y. 1996. Siembra de olivos en el desierto palestino. En: Tropical Agriculture, http://agrotropical.edunet.es. 25 p.; accessed: November 2003.

Patents: Author(s). Year. Title. Patent country and number. Retrieved from. Example: Glenn RW. 1996. Liquid personal cleansing compositions which contain soluble oils and soluble synthetic surfactants. U.S. Patent No. 6194364. Retrieved from: https://patents.google.com/patent/US6194364B1/en

ÉTICA EN LA PUBLICACIÓN CIENTÍFICA Y ACUERDO SOBRE POSIBLES MALAS PRÁCTICAS

La revista Facultad Nacional de Agronomía espera y verificará que los autores, revisores, editores y en general la comunidad académica y científica involucrada en nuestro proceso editorial, sigan estrictamente las normas éticas internacionales requeridas en el proceso de edición.

La revista Facultad Nacional de Agronomía sigue las normas éticas presentes en el COPE Best Practice Guidelines for Journal Editors y por el International Standars for Editors and Authors publicado por Committee on Publication Ethics.

Los autores deben evitar incurrir al plagio de la información. La revista define los siguientes lineamientos, criterios y recomendaciones sobre la ética en la publicación científica:

1. Criterios generales[1]

1.1. Los artículos deben contener suficiente detalle y referencias que permitan replicar o rebatir el estudio.

1.2.Declaraciones fraudulentas o deliberadamente inexactas constituyen un comportamiento poco ético.

1.3. Si el estudio incluye productos químicos, procedimientos o equipos que tienen cualquier riesgo inusual inherente a su uso, el autor debe identificar claramente estos en el artículo.

1.4. Si el estudio implica el uso de animales o de seres humanos, el autor debe asegurarse que el artículo contenga una declaración que haga explícito que se realizaron todos los procedimientos de conformidad con las leyes y directrices institucionales.

1.5. Se deben respetar los derechos de privacidad de los seres humanos.

2. Autoría[2]

Criterios:

2.1. Un "autor" es la persona que ha hecho una contribución intelectual significativa al artículo, por lo tanto, todas las personas nombradas como autores deben reunir los requisitos de autoría, y todos aquellos que los reúnan deben ser mencionados de forma explícita.

2.2. Se deben cumplir colectivamente tres criterios básicos para ser reconocido como autor:

a) Contribución sustancial a la concepción y diseño, adquisición de datos, análisis e interpretación del estudio.

b) Redacción o revisión del contenido intelectual.

c) Aprobación de la versión final.

2.3. El orden de la autoría debe ser una decisión conjunta de los coautores.

2.4. Las personas que participen en un estudio pero que no se ajusten a los criterios de autoría deben aparecer como "Colaboradores" o "Personas reconocidas".

2.5. Hay tres tipos de autorías que se consideran inaceptables: autores "fantasma", que contribuyen sustancialmente pero no son reconocidos (a menudo pagados por promotores comerciales); autores "invitados", que no hacen ninguna contribución discernible pero se nombran para aumentar las posibilidades de publicación; y autorías "honorarias", que se basan únicamente en una afiliación tenue con un estudio.

Recomendaciones:

2.6. Antes de iniciar la investigación se recomienda documentar la función y la forma como se reconocerá la autoría de cada investigador.

2.7. No se debe mentir sobre la participación de una persona en la investigación o publicación, si su contribución se considerada "sustancial" se justifica la autoría, bien sea como coautor o colaborador.

2.8. No se debe asignar una autoría sin contar con el consentimiento de la persona.

2.9. Todas las personas nombradas como autores deben reunir los requisitos de autoría, y todos aquellos que reúnan los requisitos deben aparecer como autores o contribuidores.

2.10. Algunos grupos colocan los autores por orden alfabético, a veces con una nota para explicar que todos los autores hicieron contribuciones iguales al estudio y la publicación.

3. Cambios en la autoría[3]

Criterios:

3.1. Hace referencia a la adición, supresión o reorganización de los nombres de autor en la autoría de un artículo aceptado.

3.2. Las peticiones de añadir o eliminar un autor, o para reorganizar los nombres de los autores, deben ser enviados por el autor correspondiente del artículo aceptado, y deben incluir:

a) La razón por la cual debe ser añadido o eliminado, o los nombres de los autores reorganizado.

b) La confirmación por escrito (e-mail) de todos los autores que están de acuerdo con la adición, supresión o reorganización. En el caso de adición o eliminación de los autores,esto incluye la confirmación de que el autor sea añadido o eliminado.

4. Conflicto de intereses[4]

Criterios:

4.1. Cuando un investigador o autor, editor tenga alguna opinión o interés financiero/personal que pueda afectar su objetividad o influir de manera inapropiada en sus actos, existe un posible conflicto de intereses. Este tipo de conflictos pueden ser reales o potenciales.

4.2. Los conflictos de intereses más evidentes son las relaciones financieras, como:

a) Directas: empleo, propiedad de acciones, becas, patentes.

b) Indirectas: honorarios, asesorías a organizaciones promotoras, la propiedad de fondos de inversión, testimonio experto pagado.

4.3. Los conflictos también pueden existir como resultado de relaciones personales, la competencia académica y la pasión intelectual. Por ejemplo, un investigador que tenga:

a) Algún tipo de interés personal en los resultados de la investigación.

b) Opiniones personales que están en conflicto directo con el tema que esté investigando.

Recomendaciones:

4.4. Revelar si se está en algún conflicto real o potencial de intereses que influya de forma inapropiada en los hallazgoso resultados del trabajo presentado, dentro de los tres (3) años de haber empezado el trabajo presentado que podría influir indebidamente (sesgo) el trabajo.

4.5. Revelar el papel de un promotor (o promotores) del estudio, si los hubiere, en el diseño del estudio, en la recopilación, análisis e interpretación de los datos, en la redacción del informe y en la decisión de presentar el documento para su publicación.

4.6. Los investigadores no deben entrar en acuerdos que interfieran con su acceso a todos los datos y su capacidad de analizarlos de forma independiente, y de preparar y publicar los manuscritos.

4.7. Al presentar un documento, se debe hacer una declaración (con el encabezamiento "Papel que ha tenido la fuente de financiación") en una sección separada del texto y colocarse antes de la sección "Referencias".

4.8. Algunos ejemplos de posibles conflictos de intereses que deben ser revelados, incluyen: empleo, consultoría, propiedad de acciones, honorarios, testimonio experto remunerado, las solicitudes de patentes / registros y subvenciones u otras financiaciones.

4.9. Todas las fuentes de apoyo financiero para el proyecto deben ser revelados.

4.10. Se debe describir el papel del patrocinador del estudio.

5. Publicación duplicada[5]

Criterios:

5.1. Los autores tienen la obligación de comprobar que su artículo sea basado en una investigación original (nunca publicada anteriormente). El envío o reenvío intencional de su trabajo para una publicación duplicada se considera un incumplimiento de la ética editorial.

5.2. Se produce una publicación duplicada o múltiple cuando dos o más artículos, sin hacerse referencias entre sí, comparten esencialmente las

mismas hipótesis, datos, puntos de discusión y/o conclusiones. Esto puede ocurrir en diferentes grados: Duplicación literal, duplicación parcial pero sustancial o incluso duplicación mediante parafraseo.
5.3. Uno de los principales motivos por los que la publicación duplicada de investigaciones originales se considera no ético es porque puede dar lugar a una "ponderación inadecuada o a un doble recuento involuntario" de los resultados de un estudio único, lo que distorsiona las pruebas disponibles.

Recomendaciones:

5.4. Los artículos enviados para su publicación deberán ser originales y no deberán haberse enviado a otra editorial. En el momento del envío, los autores deberán revelar los detalles de los artículos relacionados (también cuando estén en otro idioma), artículos similares en prensa y traducciones.
5.5. Aunque un artículo enviado esté siendo revisado y no conozca el estado, espere a que la editorial le diga algo antes de ponerse en contacto con otra revista, y sólo si la otra editorial no publicará el artículo.
5.6. Evite enviar un artículo previamente publicado a otra revista.
5.7. Evite enviar artículos que describan esencialmente la misma investigación a más de una revista.
5.8. Indique siempre los envíos anteriores (incluidas las presentaciones de reuniones y la inclusión de resultados en registros) que pudieran considerarse una publicación duplicada.
5.9. Evite escribir sobre su propia investigación en dos o más artículos desde diferentes ángulos o sobre diferentes aspectos de la investigación sin mencionar el artículo original.
5.10. Se considera manipulador crear varias publicaciones a raíz de la misma investigación.
5.11. Si desea enviar su artículo a una revista que se publica en un país diferente o en un idioma diferente, pregúntaselo a la editorial si se puede hacer esto.
5.12. En el momento del envío, indique todos los detalles de artículos relacionados en un idioma diferente y las traducciones existentes.

6. Reconocimiento de las fuentes

Criterios:

6.1. Los autores deben citar las publicaciones que han sido influyentes en la determinación de la naturaleza del trabajo presentado.
6.2. Información obtenida de forma privada, no debe ser usada sin explícito permiso escrito de la fuente.
6.3. La reutilización de las tablas y / o figuras requiere del permiso del autor y editor, y debe mencionarse de manera adecuada en la leyenda de la tabla o figura.
6.4. La información obtenida en el transcurso de servicios confidenciales, tales como manuscritos arbitrales o las solicitudes de subvención, no debe ser utilizada sin el permiso explícito y por escrito del autor de la obra involucrada en dichos servicios.

7. Fraude científico[6]

Criterios:

7.1. El fraude en la publicación científica hace referencia a la presentación de datos o conclusiones falsas que no fueron generados a través de un proceso riguroso de investigación.
7.2. Existen los siguientes tipos de fraude en la publicación de resultados de investigación:
a) Fabricación de datos. Inventar datos y resultados de investigación para después comunicarlos.
b) Falsificación de datos. La manipulación de materiales de investigación, imágenes, datos, equipo o procesos.
La falsificación incluye la modificación u omisión de datos o resultados de tal forma que la investigación no se representa de manera precisa. Una persona podría falsificar datos para adecuarla al resultado final deseado de un estudio.

Recomendaciones:

7.3. Antes de enviar un artículo, lea cuidadosamente las políticas editoriales y de datos de la revista.
7.4. Nunca modifique, cambie u omita datos de forma intencional. Esto incluye materiales de investigación, procesos, equipos, tablas, citas y referencias bibliográficas.
7.5. Tanto la fabricación como la falsificación de datos son formas de conducta incorrecta graves porque ambas resultan en publicaciones científicas que no reflejan con precisión la verdad observada.
7.6. El autor debe hacer una gestión adecuada de los datos que soportan la investigación, teniendo especial cuidado en la recopilación, producción, conservación, análisis y comunicación de los datos.
7.7. Mantenga registros minuciosos de los datos en bruto, los cuales deberán ser accesibles en caso de que un editor los solicite incluso después de publicado el artículo.

8. Plagio[7]

Criterios:

8.1. El plagio es una de las formas más comunes de conducta incorrecta en las publicaciones, sucede cuando uno de los autores hace pasar como propio el trabajo de otros sin permiso, mención o reconocimiento. El plagio se presenta bajo formas diferentes, desde la copia literal hasta el parafraseado del trabajo de otra persona, incluyendo: datos, ideas, conceptos, palabras y frases.
8.2. El plagio tiene diferentes niveles de gravedad, como por ejemplo:
a) Qué cantidad del trabajo de otra persona se tomó (varias líneas, párrafos, páginas, todo el artículo)
b) Qué es lo que se copió (resultados, métodos o sección de introducción).
8.3. El plagio en todas sus formas constituye una conducta no ética editorial y es inaceptable.
8.4. La copia literal solo es aceptable si indica la fuente e incluye el texto copiado entre comillas.

Recomendaciones:

8.5. Recuerde siempre que es esencial reconocer el trabajo de otros (incluidos el trabajo de su asesor o su propio trabajo previo) como parte del proceso.
8.6. No reproduzca un trabajo palabra por palabra, en su totalidad o en parte, sin permiso y mención de la fuente original.
8.7. Mantenga un registro de las fuentes que utiliza al investigar y dónde las utilizó en su artículo.
8.8. Asegúrese de reconocer completamente y citar de forma adecuada la fuente original en su artículo.
8.9. Incluso cuando haga referencia a la fuente, evite utilizar el trabajo de otras personas palabra por palabra salvo que lo haga entre comillas.
8.10. El parafraseado solo es aceptable si indica correctamente la fuente y se asegura de no cambiar el significado de la intención de la fuente.
8.11. Incluya entre comillas y cite todo el contenido que haya tomado de una fuente publicada anteriormente, incluso si lo está diciendo con sus propias palabras.

9. Fragmentación[8]

Criterios:

9.1. La fragmentación consiste en dividir o segmentar un estudio grande en dos o más publicaciones.
9.2. Como norma general, con tal de que los "fragmentos" de un estudio dividido compartan las mismas hipótesis, población y métodos, no se considera una práctica aceptable.
9.3. El mismo "fragmento" no se debe publicar nunca másde una vez. El motivo es que la fragmentación puede dar lugar a una distorsión de la literatura haciendo creer equivocadamente a los lectores que los datos presentados en cada fragmento (es decir, artículo de revista) se derivan de una muestra de sujetos diferente. Esto no solamente sesga la "base de datos científica", sino que crea repetición que hace perder el tiempo de los editores y revisores, que deben ocuparse de cada trabajo por separado. Además, se infla injustamente el número de referencias donde aparece citado el autor.

Recomendaciones:

9.4. Evite dividir inapropiadamente los datos de un solo estudio en dos o más trabajos.
9.5. Cuando presente un trabajo, sea transparente. Envíe copias de los manuscritos estrechamente relacionados al manuscrito en

cuestión. Esto incluye manuscritos publicados, enviados recientemente o ya aceptados.

10. Consentimiento informado

Criterios:

10.1. Los estudios sobre pacientes o voluntarios requieren la aprobación de un comité de ética.

10.2. El consentimiento informado debe estar debidamente documentado.

10.3. Los permisos y las liberaciones deben ser obtenidos, cuando un autor desea incluir detalles de caso u otra información personal o imágenes de los pacientes y cualquier otra persona.

10.4. Especial cuidado debe tenerse con la obtención del consentimiento respecto a los niños (en particular cuando un niño tiene necesidades especiales o problemas de aprendizaje), donde aparece la cabeza o la cara de una persona, o cuando se hace referencia al nombre de un individuo u otros datos personales.

11. Corrección de artículos publicados[9]

Criterio:

Cuando un autor descubre un error o inexactitud significativa en el trabajo publicado, es obligación del autor notificar de inmediato a la revista y cooperar en el proceso de corrección.

Referencias

Black, William, Rodolfo Russo, y David Turton. «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes». Physics Letters B 694, n.º 3 (noviembre de 2010): 246-51.

Elsevier. «Autoría. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0010/183394/ETHICS_ES_AUTH01a_updatedURL.pdf.

———. «Conflicto de intereses. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0006/183399/ETHICS_ES_COI01a_updatedURL.pdf.

———. «Envío simultáneo/múltiple, publicación duplicada. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0019/183403/ETHICS_ES_SSUB01a_updatedURL.pdf.

———. «Ethics. Conducting research». Accedido 8 de agosto de 2014. http://www.elsevier.com/journal-authors/ethics#conducting-research.

———. «Ethics. Writing an article». Accedido 8 de agosto de 2014. http://www.elsevier.com/journal-authors/ethics#writing-an-article.

———. «Fragmentación. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0018/183402/ETHICS_ES_SS01a_updatedURL.pdf.

———. «Fraude en investigación. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0017/183401/ETHICS_ES_RF01a_updatedURL.pdf.

———. «Plagio. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0016/183400/ETHICS_ES_PLA01a_updatedURL.pdf.

[1] Elsevier, «Ethics. Conducting research», accedido 8 de agosto de 2014, http://www.elsevier.com/journal-authors/ethics#conducting-research.

[2] Elsevier, «Autoría. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0010/183394/ETHICS_ES_AUTH01a_updatedURL.pdf.

[3] William Black, Rodolfo Russo, y David Turton, «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes», Physics Letters B 694, n.º 3 (noviembre de 2010): 246-51.

[4] Elsevier, «Conflicto de intereses. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0006/183399/ETHICS_ES_COI01a_updatedURL.pdf.

[5] Elsevier, «Envío simultáneo/múltiple, publicación duplicada. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0019/183403/ETHICS_ES_SSUB01a_updatedURL.pdf.

[6] Elsevier, «Fraude en investigación. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0017/183401/ETHICS_ES_RF01a_updatedURL.pdf.

[7] Elsevier, «Plagio. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0016/183400/ETHICS_ES_PLA01a_updatedURL.pdf.

[8] Elsevier, «Fragmentación. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0018/183402/ETHICS_ES_SS01a_updated updatedURL.pdf.

[9] Elsevier, «Ethics. Writing an article», accedido 8 de agosto de 2014, http://www.elsevier.com/journal-authors/ethics#writing-an-article.

PUBLICATION ETHICS AND PUBLICATION MALPRACTICE STATEMENT

The journal Revista Facultad Nacional de Agronomia follows the COPE Code of Conduct and Best Practice Guidelines for Journal Editors and the International Standards For Editors and Authors, published by Committe on Publication Ethics.
The journal puts forth the following criteria and recommendations for ethical scientific publications:

1. General criteria[1]

1.1. Articles must contain sufficient details and references that allow the study to be replicable or refutable.
1.2. Fraudulent or deliberately inexact statements constitute unethical behavior.
1.3. If a study includes the use of chemical products, procedures, or equipment that presents an inherent risk, the author must state so in the article.
1.4. If the study involves the use of animals or human beings, the article must contain a clear statement that all of the procedures were carried out in strict compliance with laws and institutional directives.
1.5. The privacy of the human beings must be respected.

2. Authorship[2]

Criteria:
2.1. An "author" is a person that has made a significant intellectual contribution to an article; all of the individuals that are named as authors must fulfill the requirements for authorship and all of those individuals that do so must be explicitly named.
2.2. Three basic criteria must be met in order to be considered an author:
a) Substantial contribution to the study concept, design, and data collection, analysis and interpretation.
b) Revision of the intellectual content.
c) Approval of the final version.
2.3. The order of the author list must be a joint decision of the coauthors.
2.4. The individuals that participate in a study but that do not meet the criteria for authorship must be listed as an "Assistant" or "recognized person."
2.5. There are three types of unacceptable authorship: "ghost" authors, who make a substantial contribution but are not recognized (often paid by commercial promoters); "guest" authors, who do not make a discernable contribution but are named in order to increase the probability of publication; and "honorary" authors, who only have a tenuous connection to the study.
Recommendations:
2.6. Before starting the research, establish the function of each researcher and the manner in which they will be recognized.
2.7. It is not necessary to mention an individual's participation in a study or publication, but if their contribution is substantial, than authorship would be justified, either as an author or assistant.
2.8. Authorship cannot be bestowed on an individual without their consent.
2.9. All of the individuals that are named as authors must meet the requirements for authorship and all of those that meet the requirements must appear as authors or assistants.
2.10. Some groups list the authors alphabetically, sometimes with a notation that indicates that all of the authors contributed equally to the study and the publication.

3. Changes in the authorship[3]

Criteria:
3.1. Additions to, removals from, and reorganization of the author names in accepted articles must be noted.
3.2. Petitions to add to, remove from, or reorganize the authors must be sent by the corresponding author of the accepted articles and must include:
a) The reason for the addition, elimination, or reorganization.
b) A written statement (e-mail) from all of the authors that confirms their agreement with the addition, elimination, or reorganization. In the case of an addition or elimination, a confirmation is also required from the author to be added or removed.

4. Conflict of interest[4]

Criteria:
4.1. When a researcher or author has a financial/personal opinion or interest that could affect their objectivity or improperly influence their actions, there exists a possible conflict of interest. Conflicts can be actual or potential.
4.2. The most evident conflicts of interest are financial, such as:
a) Direct: employment, stocks, scholarships, patents.
b) Indirect: assistantship to promoting organizations, investment funds, paid expert testimony.
4.3. Conflicts can also arise from personal relationships, academic competition, and intellectual passion. For example, an author could have:
a) Some personal interest in the results of the research.
b) Personal opinions that are in direct conflict with the research topic.
Recommendations:
4.4. Disclose all conflicts of interest, actual or potential, that inappropriately influence the findings or results of a study, including any that arise within the three (3) years after the start of said study if they could unduly (bias) influence the study.
4.5. Disclose the role of any promoter (or promoters) in the study, if any, in the design, in the collection, analysis or interpretation of the data, in the document review, or in the decision to present the document for publication.
4.6. The researchers must not enter into agreements that interfere with their access to all of the data or with their ability to independently analyze the data or to prepare and publish the manuscript.
4.7. The document must contain a statement (with the heading "Role of the financial source") in a section that is separate from the text and before the References section.
4.8. Some examples of conflicts of interest that must be revealed include: employment, consulting, stocks, honorariums, paid expert testimony, patent requests or registration, and subsidies or other financing.
4.9. All of the sources of financial support for the project must be revealed.
4.10. The role of any study sponsors must be described.

5. Duplicate publication[5]

Criteria:
5.1. Authors have the obligation of proving that their article is based on original research (never before published). The intentional submission or resubmission of a manuscript for duplicate publication is considered a breach of editorial ethics.
5.2. A duplication publication, or multiple publication, results when two or more articles, without any reference to each other, essentially share the same hypothesis, data, discussion points, and/or conclusions. This can occur to different degrees: literal duplication, partial but substantial duplication or paraphrasal duplication.
5.3. One of the main reasons that duplicate publications are considered unethical is that they can result in the "inappropriate weighting or unwitting double counting" of results from just one study, which distorts the available evidence.
Recommendations:
5.4. Articles sent for publication must be original and not sent to other editors. When sent, the authors must reveal the details of related articles (even when in another language) and similar articles being printed or translated.

5.5. Even though a submitted article is being reviewed and the final decision is not known, wait to receive notification from the editors before contacting other journals and then only do so if the editors decline to publish the article.
5.6. Avoid submitting a previously published article to another journal.
5.7. Avoid submitting articles that essentially describe the same research to more than one journal.
5.8. Always indicate previous submissions (including presentations and recorded results) that could be considered duplicate results.
5.9. Avoid writing about your research in two or more articles from different angles or on different aspects of the research without mentioning the original article.
5.10. Creating various publications based on the same research is considered a type of manipulation.
5.11. If an author wishes to send an article to a journal that is published in a different country or a different language, ask for permission from the editors first.
5.12. When submitting an article, indicate all of the details of the article that were presented in a different language along with the relevant translations.

6. Acknowledging sources

Criteria:

6.1. Authors must cite the publications that had an influence on the determination of the nature of the offered study.
6.2. Privately obtained information cannot be used without the express written consent of the source.
6.3. Republishing tables or figures requires the permission of the author or editor, who must be appropriately cited in the table or figure legend.
6.4. Information obtained through confidential services, such as arbitration articles or subsidy applications, cannot be used without the express written consent of the author of the work involved in said services.

7. Scientific fraud[6]

Criteria:

7.1. Fraud in scientific publications refers to the presentation of false data or conclusions that were not obtained through a rigorous research process.
7.2. The following types of fraud exist for the publication of research results:
a) Fabricating data. Inventing research data and results for later dissemination.
b) Falsification of data. The manipulation of research material, images, data, equipment or processes. Falsification includes the modification or omission of data or results in such a way that the research is not represented in a precise manner. A person may falsify data in order to obtain the desired final results of a study.

Recommendations:

7.3. Before submitting an article, carefully read the editorial and data policies of the journal.
7.4. Never modify, change or omit data intentionally. This includes research material, processes, equipment, tables, citations, and bibliographical references.
7.5. Fabricating and falsifying data constitute grave misconduct because both result in scientific publications that do not precisely reflect the actual observations.
7.6. Authors must appropriately manage the data that supports the research, taking special care in the compilation, production, preservation, analysis and presentation of the data.
7.7. Maintain precise records of the raw data, which must be assessable in case the editors request them after publication of the article.

8. Plagiarism[7]

Criteria:

8.1. Plagiarism is one of the more common types of misconduct in publications; it occurs when an author passes the work of others off as their own without permission, citations, or acknowledgment. Plagiarism can occur in different forms, from literally copying to paraphrasing the work of another person, including data, ideas, concepts, paragraphs, and phrases.
8.2. Plagiarism has different degrees of severity; for example:
a) The quantity of work taken from another person (various lines, paragraphs, pages, or the entire article).
b) What is copied (results, methods, or introduction section).
8.3. Plagiarism, in all of its forms, constitutes unethical behavior and is unacceptable.
8.4. Literal copying is acceptable if the source is indicated and the text is placed in quotation marks.

Recommendations:

8.5. Always remember that it is vital to recognize the work of others (including the work of your assistants or your previous studies).
8.6. Do not reproduce the work of others word for word, in totality or partially, without the permission and recognition of the original source.
8.7. Maintain a record of the sources that are used in the research and where they are used in the article.
8.8. Be sure to accurately acknowledge and cite the original source in your article.
8.9. Even when referencing the source, avoid using the work of others word for word unless it is placed in quotations.
8.10. Paraphrasing is only acceptable if the source is correctly indicated and the source's intended meaning is not changed.
8.11. Use quotations, and cite all of the content that is taken from a previously published source even when using your own words.

9. Fragmentation[8]

Criteria:

9.1.Fragmentation occurs when a large study is divided or segmented into two or more publications.
9.2. As a general rule, as long as the "fragments" of a divided study share the same hypothesis, populations, and methods, this not considered an acceptable practice.
9.3. The same "fragment" can never be published more than one time. Fragmentation can result in distortion of the literature, creating the mistaken belief in readers that the data presented in each fragment (i.e. journal article) are derived from different subject samplings. This not only distorts the "scientific database", but creates repetition that results in a loss of time for editors and evaluators that must work on each article separately. Furthermore, the cited author receives an unfair increase in their number of references.

Recommendations:

9.4. Avoid inappropriately dividing the data of one study into two or more articles.
9.5. When presenting your work, be transparent. Send copies of the manuscripts that are closely related to the manuscript in question, including published, recently submitted and accepted manuscripts.

10. Informed consent

Criteria:

10.1. Studies on patients and volunteers require the approval of the ethics committee.
10.2. The informed consent must be duly documented.
10.3. Permission and waivers must be obtained when an author wishes to include details of a case or other personal information or images of the patients or any other person.
10.4. Special care should be taken when obtaining the consent

of children (especially when a child has special needs or learning disabilities) when their head or face is displayed or when reference is made to the name of an individual or other personal data.

11. Correction of published articles[9]

Criterion:

When an author discovers a significant inexactitude or error in a published article, they must immediately notify the journal and cooperate in the correction process.

References

Black, William, Rodolfo Russo, y David Turton. «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes». *Physics Letters B* 694, n.º 3 (noviembre de 2010): 246-51.

Elsevier. «Autoría. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0010/183394/ETHICS_ES_AUTH01a_updatedURL.pdf.

———. «Conflicto de intereses. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0006/183399/ETHICS_ES_COI01a_updatedURL.pdf.

———. «Envío simultáneo/múltiple, publicación duplicada. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0019/183403/ETHICS_ES_SSUB01a_updatedURL.pdf.

———. «Ethics. Conducting research». Accedido 8 de agosto de 2014. http://www.elsevier.com/journal-authors/ethics#conducting-research.

———. «Ethics. Writing an article». Accedido 8 de agosto de 2014. http://www.elsevier.com/journal-authors/ethics#writing-an-article.

———. «Fragmentación. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0018/183402/ETHICS_ES_SS01a_updatedURL.pdf.

———. «Fraude en investigación. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0017/183401/ETHICS_ES_RF01a_updatedURL.pdf.

———. «Plagio. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0016/183400/ETHICS_ES_PLA01a_updatedURL.pdf.

---

[1] Elsevier, «Ethics. Conducting research», accedido 8 de agosto de 2014, http://www.elsevier.com/journal-authors/ethics#conducting-research.

[2] Elsevier, «Autoría. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0010/183394/ETHICS_ES_AUTH01a_updatedURL.pdf.

[3] William Black, Rodolfo Russo, y David Turton, «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes», *Physics Letters B* 694, n.º 3 (noviembre de 2010): 246-51.

[4] Elsevier, «Conflicto de intereses. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0006/183399/ETHICS_ES_COI01a_updatedURL.pdf.

[5] Elsevier, «Envío simultáneo/múltiple, publicación duplicada. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0019/183403/ETHICS_ES_SSUB01a_updatedURL.pdf.

[6] Elsevier, «Fraude en investigación. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0017/183401/ETHICS_ES_RF01a_updatedURL.pdf.

[7] Elsevier, «Plagio. Ethics in research & publication», accedido de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0016/183400/ETHICS_ES_PLA01a_updatedURL.pdf.

[8] Elsevier, «Fragmentación. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0018/183402/ETHICS_ES_SS01a_updated updatedURL.pdf.

[9] Elsevier, «Ethics. Writing an article», accedido 8 de agosto de 2014, http://www.elsevier.com/journal-authors/ethics#writing-an-article.