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Índice Bibliográfico Nacional
Colciencias - Publindex

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8553 **Phytophagous insects in tamarind crop with emphasis on those causing fruit damage in the nearby Western of Antioquia**
Insectos fitófagos en el cultivo de tamarindo con énfasis en los que causan daño al fruto en el Occidente cercano Antioqueño

Mariana Mercado-Mesa / Verónica M. Álvarez-Osorio / Jhon Alveiro Quiroz /Sandra B. Muriel

8563 **Antifungal activity of extracts, essential oil and constituents from *Petroselinum crispum* against *Colletotrichum acutatum***
Actividad antifúngica de extractos, aceite esencial y constituyentes de *Petroselinum crispum* contra *Colletotrichum acutatum*

Rodrigo Pineda / Samuel Vizcaíno / Carlos M. García / Jesús H. Gil / Diego Durango

8573 **Common bean (*Phaseolus vulgaris* L.) yield response to chemical and biological fertilization in different localities of Colombia**
Respuesta productiva del cultivo del fríjol (*Phaseolus vulgaris* L.) a la fertilización química y biológica en zonas productoras de Colombia

Álvaro Tamayo Vélez / Jorge Alonso Bernal Estrada

8581 **Determination of predominant soluble salts in soils of the irrigation district Alto Chicamocha of Boyacá**
Determinación de sales solubles predominantes en suelos del distrito de riego Alto Chicamocha de Boyacá

Ingrid Walteros Torres / Germán Cely Reyes / Diego Moreno Pérez

8593 **Comparison of soil use in the infiltration of rainwater: pasture and forest**
Comparación del uso del suelo en la infiltración del agua de lluvia: pastos y bosque

Edson Lopes / Rosemeri Carvalho Marenzi / Tito César Marques de Almeida

8601 **Effects of land-use change on Nitisols properties in a tropical climate**
Efectos del cambio de uso de la tierra en las propiedades de los Nitisoles en un clima tropical

Dilier Olivera Viciedo / Alberto Hernández / Manuel Rodríguez / Rodolfo Lizcano / Alexander Calero / Kolima Peña

8609 **Bioclimatic analysis of three buildings for wet processing of coffee in Colombia**
Análisis bioclimático de tres instalaciones para el beneficio húmedo de café en Colombia

Robinson Osorio Hernández / Ilda de Fatima Ferreira Tinôco / Joyce Correna Carlo / Jairo Alexander Osorio Saraz / Iván Darío Aristizábal Torres

8617 **Effect of drying methods on physical and chemical properties of *Ilex guayusa* leaves**
Efecto de métodos de secado sobre las propiedades físicas y químicas de las hojas de *Ilex guayusa*

Patricia Manzano Santana / María Quijano-Avilés / Iván Chóez-Guaranda / Ana Barragán Lucas / Rafael Viteri Espinoza / Daysi Martínez / Cinthia Camacho / Migdalia Miranda Martinez

8623 **Satisfaction among dairy farm owners after certification on good management practices**
Satisfacción entre propietarios de granjas lecheras luego de certificarse en buenas prácticas ganaderas

Brayian Cuartas Martínez / Dursun Barrios / Mario Fernando Cerón-Muñoz

8631 **Pysicochemical properties and application of edible coatings in strawberry (*Fragaria* x *Ananassa*) preservation**
Propiedades fisicoquímicas y aplicación de recubrimientos comestibles en la conservación de fresa (*Fragaria* x *Ananassa*)

Sandra G. Barrazueta-Rojas / José F. Falcon / Marcelo N. Navarro Ojeda / Julio M. Oleas-López / Guillermo X. Mendoza-Zurita

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.

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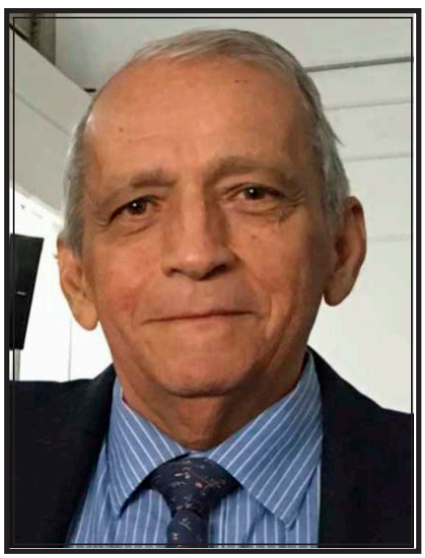
RODRIGO ANTONIO VERGARA RUIZ

(1945 - 2017)



Rodrigo Vergara Ruiz nació en el municipio de Anorí, Antioquia el 17 de mayo de 1945 y murió en la ciudad de Medellín el 28 de diciembre de 2017. Cursó el bachillerato en la Universidad de Antioquia y se graduó de Ingeniero Agrónomo en la Universidad Nacional de Colombia, Sede Medellín, en 1971. Adelantó estudios de posgrado como Especialista en Docencia de la Biología en la Universidad del Tolima - Ibagué: 1991-1992 e hizo una Maestría en Ciencias de la Entomología en el ICA, Instituto Colombiano Agropecuario, programa PEG - Universidad Nacional de Colombia – Bogotá.

Su padre, José Vergara Calle, educó a sus 10 hijos trabajando detrás del mostrador de su tienda "El Prodigio", así se llamaba su lugar de trabajo en el municipio de Anorí, en unión de su esposa Carmelina Ruiz.

Hombre creyente y brillante profesional de las Ciencias Entomológicas, con una memoria prodigiosa; pronunciaba en sus conferencias decenas de palabras científicas de insectos sin ninguna vacilación, así como los nombres de científicos e investigadores a los que se refería para respaldar sus conceptos y ponencias. Fue un gran colaborador del gremio de profesionales del sector agrario del país.

Como miembro de la junta directiva de ASECA_UN (Asociación de Egresados de la Facultad de Ciencias Agrarias de la Universidad Nacional de Colombia, Sede Medellín), el profesor Vergara Ruiz hizo su última intervención académica en Corporación Colombiana de Investigación Agropecuaria –AGROSAVIA- C.I. La Selva, en el municipio de Rionegro Antioquia, con la conferencia "Insectos Plagas y Enfermedades en Pastos. Manejo y Control Biológico", dentro del curso "Enfoque y Manejo Ecológico de la Ganadería", organizado por ASECA-UN durante los días 9 y 10 de noviembre de 2017, prácticamente un mes antes de su fallecimiento.

Los reconocidos entomólogos José Iván Zuluaga C. y Francisco Yepes R., profesores de la Universidad Nacional de Colombia, se refieren a él como "Maestro de la enseñanza y la divulgación entomológica en Colombia".

Docencia y actividades académicas

El profesor Vergara Ruiz se inició como docente universitario en la Facultad de Agronomía de la Universidad Pedagógica y Tecnológica de Colombia - UPTC en la ciudad de Tunja, capital del departamento de Boyacá, durante el periodo 1971 a 1988. Fue director del Centro de Investigaciones Agropecuarias INIAG-UPTC (1981-1984). Se desempeñó como Profesor visitante en la Universidad del Tolima (1988 a 1989), en su capital Ibagué. Posteriormente se vinculó como Profesor Asociado en el Departamento de Agronomía de la Universidad Nacional de Colombia, Sede Medellín; Facultad de Ciencias Agropecuarias, hoy Facultad de Ciencias Agrarias, entre 1992 y 2004.

Rodrigo Vergara realizó importantes aportes en el campo de la Extensión Universitaria y en el de las asociaciones de profesionales agrarios; además, prestó asesoría fitosanitaria en unión con reconocidos fitopatólogos como Pablo Buriticá Céspedes y José J. Castaño Arenas. Debo mencionar su amistad con el médico toxicólogo Dario Córdoba P., quien fuera Asesor del Consejo Seccional de Plaguicidas de Antioquia, con quien trabajó el tema de la toxicología en condiciones de campo con importantes instituciones líderes del sector agropecuario y campesinos. Como tal, fue un activo promotor y conferencista en eventos tales como Simposios, Congresos de Entomología y Ecología, Foros y también en Talleres y Seminarios Entomológicos, con énfasis en manejo de plagas de importancia económica.

Participó activamente en los Congresos Anuales de Socolen (Sociedad Colombiana de Entomología), en los Simposios Nacionales de Control Biológico, en los Foros Entomológicos de la Facultad de Ciencias Agropecuarias, Sede Palmira, en Simposios Inter-

nacionales y Nacionales sobre el impacto de los plaguicidas en la salud humana y el ambiente, organizados por Rapalmira (Red Mundial de plaguicidas, en la Universidad Nacional de Colombia Sede Palmira). Promovió en unión de profesores y estudiantes, los tradicionales Seminarios anuales del GEUN, Grupo de Estudios entomológicos de la Universidad Nacional de Colombia).

Se resalta dentro de su discurrir profesoral universitario la dirección de trabajos de investigación y seminarios, tesis de pregrado y posgrado, y un importante número de publicaciones personales, con colegas y estudiantes. Se contabilizan dentro de su producción académica: 9 libros, aproximadamente 243 artículos publicados y otros trabajos de promoción, que hoy son referentes dentro de la lectura de la entomología Colombiana, según los entomólogos: José Iván Zuluaga C. y Francisco C. Yepes R., profesores de la Universidad Nacional de Colombia. En suma, fueron 46 años de intensa actividad académica.

Actividades administrativas
En este campo fue muy diligente, se inició como Director del Centro de Investigaciones del Departamento de Producción y Sanidad Vegetal en la Facultad de Ciencias Agropecuarias de la Universidad Nacional de Colombia, Sede Medellín, (hoy Facultad de Ciencias Agrarias), en donde ocupó el cargo de Director del Departamento de Agronomía (1993 -1994).

Fue designado como decano de la misma Facultad, durante el periodo abril de 1994 hasta abril de 1996.

Ocupó la Vicepresidencia de la junta directiva de Socolen durante el periodo 2014 - 2016.

Fue además, miembro permanente del Consejo Regional de Plaguicidas de Antioquia y ponente en sus respectivos seminarios. Participó como conferencista en los dos primeros eventos de “Insectópolis” efectuados en la ciudad de Cali. Fue integrante del Comité Nacional de Control Biológico, grupo auspiciado por Colciencias.

Distinciones y Reconocimientos
Fue Reconocido con 17 distinciones honoríficas tanto universitarias como de las asociaciones de las que fue socio y miembro activo; contó con la dedicación de dos especímenes de insectos que llevan su nombre, así como muchas menciones con que se le distinguió. En 1983 recibió la de Ingeniero Agrónomo del año. Se resaltó su trabajo profesional con numerosos diplomas, pergaminos, placas y medallas.

La Facultad de Ciencias Agrarias de la Universidad Nacional de Colombia, Sede Medellín y la Oficina de Egresados, lo reconoció como uno de sus graduados Ilustres, distinción que se entregó durante la celebración de los cien años de existencia de la Facultad en abril de 2014.

La doctora Lilliam Eugenia Gómez Álvarez, presidente del Consejo Seccional de Plaguicidas de Antioquia, escribió el 29 de diciembre de 2017, día de su fallecimiento: “Gracias Rodrigo, compañero y amigo de luchas de este consejo, por el sueño de un mundo libre de xenobióticos ¡Qué hoy, la tierra te sea ligera…!

Desde nuestros corazones un adiós a tu energía en algún lugar ignoto, donde quiera que ella se encuentre.

Gustavo Jiménez Narváez I.A. EF.
Coordinador Egresados Facultad de Ciencias Agrarias
de la Universidad Nacional de Colombia
Sede Medellín

YOAV BASHAN

(1951-2018)

Con mucho pesar registramos el fallecimiento de nuestro amigo Yoav Bashan, gran científico y humanista reconocido mundialmente. Las puertas de su laboratorio estuvieron abiertas para el intercambio de estudiantes de todas la universidades del mundo, incluyendo estudiantes de la Universidad Nacional de Colombia. Era parte del Comité Científico de esta revista, al igual que de otras revistas internacionales que se preciaban de tenerlo allí.

Esta breve reseña, elaborada a partir del sitio web de la Fundación Bashan (www.bashanfoundation.org) se muestra su perfil científico. Celebremos la vida de este gran hombre y sus aportes a la comunidad científica.

Yoav Bashan recibió el titulo de B.Sc. en Agronomía (1976), M.Sc. en Fitobacteriología (1977) y un Doctorado en Fitopatología (1983). Estos titulos los recibió en la Hebrew University of Jerusalem, Israel. Realizó pasantías postdoctorales en la Hebrew University of Jerusalem, en el Weizmann Institute of Science y en el Departmento de Agronomy de La Ohio State University.

Desde 1998 hasta el presente, se desempeñaba como el lider del Grupo en Microbiología Ambiental y Biotecnología (Environmental Microbiology and Biotechnology) en el Centro de Investigaciones Biologicas del Noroeste (CIBNOR) La Paz, Mexico. Allí tenía el rango equivalente a “Senior Distinguished Professor”.

Desde 2003 fue el Presidente y cientifico-residente de la Fundación Bashan para el Avance de la Ciencia y las Artes , Corvallis, Oregon, USA. Esta fundación es un instituto científico internacional virtual para soportar la difusión libre de información científica, la formación de estudiantes de posgrado de paises en desarrollo y las artes (www.bashanfoundation.org) Corvallis, Oregon.

Desde 2014 hasta el presente fue el presidente del Instituto de Ciencia Bashan (The Bashan Institute of Science; www.bashanis.org) donde se consolidó para desarrollar un instituto para el avance de la ciencia. Este se estableció en Auburn, Alabama, USA.

Desde 2013 hasta el presente fue Profesor afiliado al Departmento de Entomología y Patología de Plantas, Auburn. University, Alabama, USA.

Desde 2008 al presente fue profesor adjunto del Departamento de Ciencias del Suelo, Agua y Ambiente. Universidad de Arizona, Tucson, Arizona, USA.

Desde el año 2001 hasta el 2011 fue profesor adjunto en el Departmento de Ciencia del Suelo e Ingeniería Agrícola, Facultad de Agricultura y Ciencia de Alimentos, Laval University, Quebec, Canada.

Desde el año 2003 hasta el 2005 fue profesor adjunto en el Centro Interdisciplinario para las Ciencias Marinas, Instituto Politécnico Nacional, Ciudad de Mexico, Mexico.

Desde el año 1990 hasta 1997 se desempeñó como Director del Departamento de Microbiología, Centro para la Investigación Biológica, La Paz, Mexico.

Desde el año 1985 hasta 1987 se desempeñó como Cientifico- Profesor Asistente en el Deparatmento de Genética de Plantas, Weizmann Institute of Science, Rehovot, Israel.

Areas de Investigación

Sus áreas de investigación durante más de tres decadas fueron Microbiología Ambiental, Restauración Ecológica y Biotecnología. Lideró el campo de investigación sobre bacterias promotoras de crecimiento vegetal con aplicaciones en agricultura y en restauración ambiental. Igualmente, estudió sobre las interacciones planta-microalga-microbios en ambientes acuáticos y terrestres, restauración, reclamación y conservación de ecosistemas disturbados, bioremediación de agua y microbiología del suelo. Con sus proyectos restauró humedales en Mexico a su condición original y demostró métodos para la restauración de tierras erosionadas en parcelas de campo.

Hasta Enero de 2018, el professor Bashan publicó 374 trabajos cientificos, de los cuales 223 fueron publicados en revistas con revision de pares (peer-review) y con factor de impacto (el promedio del factor de impacto de los últimos 10 años fue 3305) y el resto son capitulos de libros y publicaciones de divulgación científica popular.

El professor Bashan fue miembro de comités editoriales de 17 revistas científicas de 7 países: Biology and Fertility of Soils (2004-presente) (Germany), European Journal of Soil Biology (2001-presente) (The Netherlands); Annals of Microbiology (2007-2015) (Germany); Frontiers in Microbiological Chemistry (2012) (Switzerland), The Open Forest Science Journal (2008-present) (USA), Journal of Biomedicine and Biotechnology (2009-present) (USA), Colombian Journal of Biotechnology (2002-present), Agronomía Colombiana (2008-present) (Colombia), American Biographical Institute (2002-2007) (USA), Revista Fitotecnia Mexicana (2003- 2005) (Mexico); Wildflower (Canada) (2001-2004), World Applied Sciences Journal (2006-2008) (Canada); Microbial Ecology (USA) (1998-2001), Revista Facultad Nacional de Agronomía Medellín (2016-Present) (Colombia). Además, realizó revisiones para 264 revistas científicas en 44 países y 28 agencias financiadoras de investigación en 16 países. En 2016 fue considerado por el Programa de Monitoreo de Investigación de la Unión como el científico #1 en todas las Ciencias de la Vida en Mexico. Los indicadores internacionales de productividad científica son más de 22.100 citaciones de sus publicaciones; indice h de 76 y un índice-i: 207.

Queda claro que los valores científicos de Yoav fueron muy sobresalientes. Sin duda, para aquellos que lo conocimos podemos afirmar que sus valores humanos equiparan sus logros académicos. Para nosotros en Colombia fue un maestro generoso y paciente, quien nos enseñaba y motivaba a seguir adelante con nuestras investigaciones y a apoyar a los estudiantes.

Su cercanía con Colombia fue muy estrecha, estaba casado con Luz Estella González De-Bashan, una brillante científica -integantre de nuestro Comité Editorial- quien fue su compañera de vida y de ciencia, de múltiples viajes, conferencias, proyectos de investigación y de tantos trabajos científicos. A ella le expresamos nuestro más sincero sentimiento de pesar y solidaridad.

Recomendamos a los lectores visitar la pagina web de la Fundación Bashan (www.bashanfoundation.org), allí podremos visualizar el legado de este gran hombre.

Claudia Álvarez y Walter Osorio
Universidad Nacional de Colombia
Sede Medellín

Regulación a la investigación en biodiversidad: Problemas para la microbiología agrícola

Bajo el liderazgo del Ministerio de Ambiente y Desarrollo Sostenible y en ausencia de una ley estructural, el acceso a genes y organismos de nuestra diversidad biológica, sea para investigarla o aprovecharla, se viene reglamentando desde el ejecutivo. Con Decretos y Resoluciones claramente bien intencionadas, se ha tratado de construir un marco normativo. Sin embargo, bajo premisas equivocadas para el caso de los microorganismos en general, incluidos aquellos de importancia agrícola, se ha creado una realidad legal contradictoria que limita la investigación y el aprovechamiento de nuestra diversidad microbiana.

El origen del problema parece estar en la Decisión 391 de 1996, expedida por la Comunidad Andina de Naciones. Su objeto es regular el acceso a los recursos genéticos y sus productos derivados en los países miembros del Organismo: Bolivia, Colombia, Ecuador y Perú. Las Decisión está motivada en la consideración de que "los Países miembros cuentan con un importante patrimonio biológico y genético", el cual "tiene un gran valor económico" y "debe preservarse y utilizarse de manera sostenible". Para ello, considera que "los Países miembros son soberanos en el uso y aprovechamiento de esos recursos" y trata de reconocerle un papel especial a "las comunidades indígenas, afroamericanas y locales", bajo la consideración de que los recursos en cuestión tendrían características de "endemismo y rareza" relevantes, y que esos grupos étnicos tendrían "conocimientos, innovaciones y prácticas" fundamentales para su conservación y uso sostenible.

Siendo buena la intención de la Decisión, hay, por lo menos, tres premisas equivocadas desde el punto de vista científico para el caso de los microorganismos. La primera es considerar una naturaleza "endémica y rara" de la diversidad microbiana. Por un lado, el grado de endemismo y rareza de los microorganismos es aún objeto de debate en la comunidad científica, haciendo indebido que se tome como premisa para legislar. Más aún, el grueso de esa comunidad y la evidencia disponible sugieren que, por su biología, la desenfrenada plétora de la diversidad microbiana deba ser considerada como esencialmente cosmopolita. Esto, no solo porque los patrones biogeográficos parecen ser de escala continental y no nacional, sino porque solo se han encontrado en algunos grupos. Adicionalmente, la especiación y dispersión de los microorganismos han mostrado una dinámica muy superior a la de otros seres vivos, de manera que escapan a cualquier consideración razonable de restricción geográfica estable. Así, considerar el "endemismo y rareza" como características relevantes de la diversidad biológica parece haberse basado en una reflexión exclusiva sobre la biología de plantas y animales, sin considerar las particularidades de la biología microbiana.

Otras premisas equivocadas de la Decisión 391, en el caso de los microorganismos, son la necesidad de preservar y utilizar de manera sostenible los recursos biológicos y genéticos, y considerar que las comunidades étnicas tendrían conocimientos, innovaciones y prácticas relevantes para ello. De nuevo, estas premisas parecen haber considerado exclusivamente lo que podríamos llamar la "macrobiota", y no la diversidad microbiana. Por un lado, la naturaleza de los microorganismos hace que no tenga sentido invertir esfuerzos en su preservación en ambientes naturales, pues no es razonable el riesgo de su extinción por colecta o deterioro de hábitats. Más aún, en el caso de las bacterias, cabe incluso la posibilidad de que las especies se originen y extingan constantemente, de forma natural, en una dinámica espontánea y acelerada sin final[1]. De igual forma, gracias al carácter "invisible" de los microorganismos, se hace poco convincente pensar que haya grupos étnicos con conocimientos y tradiciones dirigidas a su conservación y uso sostenible. La existencia de la vida microbiana es un descubrimiento reciente en la historia de la humanidad, además de requerir desarrollos tecnológicos avanzados para su observación y acceso.

Estas premisas, equivocadas para el mundo microbiano, han dado origen a normas igualmente equívocas cuando se aplican al ámbito de la vida microscópica. Ejemplo de ello es el Decreto 1376 de 2013, el cual reglamenta para nuestro País "los permisos de recolección de especímenes de especies silvestres de la diversidad biológica con fines de investigación científica no comercial". La primera y más evidente de las contradicciones es que este Decreto regula la recolección con fines de investigación

[1] Cohan, FM. 2016. Bacterial species concepts. En: Encyclopedia of evolutionary biology, Elsevier Inc., Volumen 1. pg 119-129.

no comercial, mientras aquella con fines comerciales, no está regulada. Como resultado, la colecta de microorganismos con fines de investigación requiere permisos del Estado, en tanto que la colecta para cualquier otro fin, incluyendo la prospección y desarrollo de productos a base de microorganismos benéficos (si se formulan como extractos crudos[2]), no los requiere. Incluso, bajo la normativa actual y estrictamente hablando, todo proceso de diagnóstico de enfermedades infecciosas en humanos, animales o plantas, siempre que requiera colecta del microorganismo (lo más frecuente), exigiría permiso, ya que todo proceso diagnóstico es un proceso esencialmente investigativo. A pesar de ello (y por fortuna), el requerimiento no se hace hoy efectivo en estos casos. Sin embargo, evidencia una contradicción fundamental, ya que acá se cumplen los mismos principios de investigaciones a las que hoy se les exige el permiso.

Sin embargo, la contradicción más profunda en el Decreto 1376 parece menos evidente: ¿Cuál puede ser el fin de regular la colecta de microorganismos con fines de investigación? ¿Por qué pedir permiso para investigarlos y no para usarlos? Si se reflexiona entorno a esto, se evidencia que dicha regulación solo toma sentido ante un riesgo de sobre-colecta del organismo, pues esto puede afectar sus poblaciones naturales y ponerlo en riesgo de extinción. Sin embargo, para el caso de los microorganismos, y como ya señalé antes, esto carece de todo sentido. Nuevamente, estas parecen ser normas bien intencionadas, pero consideradas sobre la biología de la flora y la fauna, no sobre la microbiana.

Pero la Resolución 1348 de 2014, "la cual establece las actividades que configuran acceso a los recursos genéticos y sus productos derivados para la aplicación de la Decisión Andina", es quizás la más problemática. El punto central es su imposición de cargas al investigador[3], sin beneficio real para él ni para el País. Como una forma de evitar estas cargas en el caso de investigaciones científicas básicas, el Decreto 1376 estableció que las actividades de sistemática molecular, ecología molecular, evolución y biogeografía no configuran acceso al recurso genético. Sin embargo, esta lista resulta sumamente incompleta y con frecuencia ambigua. Por ejemplo, un simple estudio genético de asociación entre un gen y un determinado producto (estudio frecuente en bacteriología) no ajusta dentro de ninguna de las excepciones y, por ende, aun siendo un estudio básico, configuraría acceso y requeriría contrato con el Estado.

Claramente, estas cargas se fundamentan en un fin loable de la Decisión 391: La búsqueda de condiciones para una participación justa y equitativa en los beneficios derivados del acceso. Sin embargo, estando basado este fin en las consideraciones revisadas arriba, queda claro que es improbable que pueda derivarse un beneficio asociado a microbiota exclusiva del País. De hecho, un gran número de investigadores recientemente manifestaron que las expectativas de la imaginación pública y de algunos gobiernos sobre el valor comercial de sus especies nativas es poco realista, toda vez que las experiencias exitosas registradas hasta el momento con productos naturales han sido derivadas de organismos ubicuos[4]. Así, el resultado final de estas cargas a la bioprospección microbiana nacional es una ventaja competitiva para los bioinsumos basados en microorganismos foráneos y que hoy entran al País o son competencia en mercados extranjeros, los cuales terminan teniendo menos trámites y costos. En otras palabras, la normativa crea una realidad en la que resulta más provechoso el desarrollo de productos con microbiota foránea que nativa, situación en clara contravía al espíritu de la Decisión 391 y en perjuicio del desarrollo del País.

Toda regulación Estatal plantea el dilema entre seguridad y libertad: Un aumento en una, conlleva un límite en la otra. Como País, nuestro interés es la conservación y aprovechamiento sostenible de nuestra biodiversidad. Para ambas, la investigación científica es fundamental y, por ende, debe ser maximizada. Para algunos segmentos de nuestra diversidad biológica, i.e. plantas o animales con algún nivel de riesgo, es razonable introducir regulaciones a la colecta. Sin embargo, en el caso de los microorganismos, esto carece de todo sentido y, por ello, regular su colecta es una carga sin beneficio. Igualmente, la investigación científica (sea básica o aplicada) no implica en sí misma un beneficio económico, por lo cual tampoco

[2] Acá hay una contradicción más. En este caso, asociada a la Resolución 1348, que reglamenta el acceso al recurso genético y sus derivados. De ella se infiere que, si un producto está basado en las moléculas separadas producidas por un microorganismo, requiere un contrato con el Estado. Por el contrario, si el producto está basado en un extracto crudo del microorganismo, no lo requiere. Desde el punto de vista científico, sin embargo, no se hace evidente ningún argumento para comprender por qué es necesario el requerimiento en el primer caso y no en el segundo.

[3] Estas cargas incluyen no solo procesos de negociación y pagos al Estado, sino trámites centralizados en Bogotá, los cuales son costos y tiempo extra para investigadores de otras regiones.

[4] K. Divakaran Prathapan y 172 firmantes de 35 países. When the cure kills-CBD limits biodiversity research. National laws fearing biopiracy squelch taxonomy studies. *Science*, Junio 29 de 2018. Número 6396: 1405-1406.

representa beneficio alguno regularla y, por el contrario, esa regulación la limita y disminuye. Desde el punto de vista de repartir beneficios, es la comercialización la que tiene sentido regular, no la investigación. Normas como las que actualmente tenemos en Colombia o las que se pueden venir con la ratificación del Protocolo de Nagoya[5] tienen efectos negativos sobre el desarrollo de investigación en microbiología agrícola en nuestro país, debido a que imponen costos sin ningún beneficio. Si aplicamos a la investigación en microorganismos el principio liberal de "tanto mercado como sea posible, tanto Estado como sea necesario", es claro que las libertades pueden ser máximas y la intervención del Estado mínima. Solo así, conservaremos y aprovecharemos al máximo nuestra biodiversidad.

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[5] Overmann, J. y Hartman, A. 2017. Microbiological research under the Nagoya protocol: Facts and fiction. *Trends in Microbiology*, 25(2): 85-88.

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Phytophagous insects in tamarind crop with emphasis on those causing fruit damage in the nearby Western of Antioquia

Insectos fitófagos en el cultivo de tamarindo con énfasis en los que causan daño al fruto en el Occidente cercano Antioqueño

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ABSTRACT

The tamarind is an important fruit for small producers of the nearby Western of Antioquia because it is offered in various presentations to tourists who visit the region. However, there are some quality problems related to the presence of insects that generate difficulties in its commercialization. The objective of this study was to determine the phytophagous insects in this tree, with emphasis on insects that cause the greatest fruit damage; in five farms of Santa Fe de Antioquia and Sopetran. The insects associated to each organ of six trees per farm were collected, each of their damage was described and they were identified as detailed as possible. Three phytophagous insects causing the greatest fruit damage were prioritized, determining their infestation percentage (IP). Therefore, a scale of damage was designed and 30 fruits per tree were evaluated. Eleven phytophagous insects associated to tamarind crop were found, five of them affecting the fruit: *Caryedon serratus*, two Phycitinae moths, *Sitophilus linearis* and *Hypothenemus obscurus*. Five new pest registers for tamarind in Colombia were reported: *H. obscurus*, *Toxoptera aurantii, Trigona* sp., *Ectomyelois ceratoniae* and, *Acromyrmex octospinosus*. The average IP value for *C. serratus*, the Phycitinae moths and, *S. linearis* were 19.5%, 8%, and, 2.5%, respectively. The first two affect the pulp and *S. linearis* affects the seed. The most frequent damaged (43% - 52%) was grade 1 while the lowest percentages (0% - 4%) corresponded to grades 4 and 5.

Keywords:
Fruit-tree
Pests
Pod quality
Infestation percentage
Damage grade

RESUMEN

El tamarindo es un fruto importante para los pequeños productores del Occidente cercano Antioqueño, en cuanto a que se ofrece en diversas presentaciones a los turistas que visitan esta región. Sin embargo, algunos problemas de calidad, relacionados con la presencia de insectos en este fruto generan dificultades para su comercialización. El objetivo de este trabajo fue determinar los insectos fitófagos de los árboles de tamarindo, con énfasis en los que causan mayor daño al fruto. En cinco fincas de Santa Fe de Antioquia y Sopetrán, se recolectaron los insectos asociados a cada órgano, de seis árboles por finca, se describió su daño y se identificaron hasta el nivel más detallado posible. Tres insectos causantes del mayor daño en el fruto tuvieron prioridad, determinándose su porcentaje de infestación (PI). Para ello, se diseñó una escala de daño y se evaluaron 30 frutos por árbol. Se encontraron once insectos fitófagos asociados al tamarindo, de los cuales cinco registros son nuevos para el tamarindo en Colombia: *H. obscurus*, *Toxoptera aurantii*, *Trigona* sp., *Ectomyelois ceratoniae* y *Acromyrmex octospinosus*. Cinco insectos atacan al fruto: *Caryedon serratus*, dos polillas Phycitinae, *Sitophilus linearis* e *Hypothenemus obscurus*. El PI promedio para *C. serratus*, las dos polillas Phycitinae y *S. linearis* fue de 19,5%, 8% y 2,5%, respectivamente. Los tres primeros afectan la pulpa y *S. linearis* ataca a la semilla. El daño más frecuente (43% - 52%) fue de grado 1 y los grados 4 y 5 se presentaron en menores porcentajes (0% - 4%).

Palabras clave:
Frutal
Plagas
Calidad de vaina
Porcentaje de infestación
Grado de daño

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Tamarind (*Tamarindus indica* L.) is an African native species (El-Siddig *et al*., 2006) adapted to semi- tropical arid conditions (Gupta *et al*. 2017). It is a very important crop for municipalities of the nearby Western of Antioquia (Colombia), since its fruit is offered in various presentations to the tourists who visit this region. Several local familiar confectionery microenterprises sell tamarind as a fresh fruit, or process the fruit to elaborate, pulps and juices. Antioquia is one of the two Colombian departments with the greatest tamarind production according to El Ministerio de Agricultura y Desarrollo Rural de Colombia - MADR (2013a), with the 30.3% of participation in the national production of this crop. In this department, the tamarind production is carried out in two municipalities of the nearby Western of Antioquia: Santa Fe de Antioquia and Sopetran, each of them with 59.9% and 40.1% of participation, respectively. The tamarind yield in the region of Antioquia (3.2 t ha^{-1}) is lower than in the Atlántico region (5.6 t ha^{-1}) (MADR, 2013b), this situation had to be analyzed with the purpose to improve the production of this fruit crop.

The tamarind fruit in the nearby western of Antioquia is obtained from dispersed trees or from traditional production systems. This fruit has low quality related to lightweight, small size, presence of insects and fungi that can discredit the products of small-scale confectionery enterprises (Correa, 2015). The most important pests of the fruit in the region, according to Muñoz and Rueda (2009) are *Cadra cautella* Walker, (Lepidoptera*:* Pyralidae) (or *Ephestia cautella), Caryedon serratus* Ol., (Coleoptera: Bruchidae) and *Sitophilus linearis* Herbst, (Coleoptera: Curculionidae). These insects cause high loss of fruits and low quality of the products elaborated with them, since, occasionally, insect parts are found in tamarind sweets (Muñoz and Rueda, 2009). In Mexico, the borer *C. serratus* is the biggest causative of damage, nevertheless, there are others Lepidoptera and Coleoptera that affect the fruit (Orozco *et al*., 2012): the spittlebug (Cercopidae), ants *Atta* and *Acromyrmex* and, the stem borer *Trachyderes mandibularis* Dupont, (Coleoptera: Cerambycidae) (Orozco, 2001; Orozco *et al*., 2009; Orozco *et al*., 2011).

Tamarind insect species of the most economic importance in India in the scale are *Aonidiella orientalis* Newstead, (Hemiptera: Diaspididae) (Patel, 2015), the mealybug *Nipaecoccus viridis* Newstead, (Hemiptera: Pseudococcidae) (Kumar, 2016) and, the fruit moth *Tophlebia ombrodelta* Lower, (Lepidoptera: Tortricidae) (Gupta *et al*., 2017). Other less important phytophagous species are *Aspidiotus destructor* Signoret, *Planococcus lilacinus* Cockerell and, *Otinotus oneratus* Walker (Butani, 1978; Ojo and Omoloye, 2015). Reports from other regions of the world show that tamarind is affected by different moths such as *Mussidia nigrivenella* Ragonot, *Ectomyelois ceratoniae* Zell., *Plodia interpunctella* Hübner, *Cadra figulilella* Gregson*, Phidotricha erigens* Ragonot (Solis, 1999), *Paralipsa gularis* Zell. (Kumar, 2016) and, *Corcyra cephalonica* Station (Devi, 2016).

The objective of this study was to determinate the phytophagous insects of the tamarind crop, focusing on those that cause the greatest fruit damage, in five farms in Santa Fe de Antioquia and Sopetran.

MATERIALS AND METHODS

Location

The study was carried out in five farms, four in the municipality of Sopetrán (6°30'21,5''N, 75°44'24,8''W) and one in the municipality of Santa Fe de Antioquia (6°33'18,35''N, 75°49,32''W) (Figure 1). These farms are located in a tropical dry forest zone (Holdridge, 1982) where the average temperature is 27 °C, the annual mean rain precipitation of 1097 mm and, the mean relative humidity is of 73.2% (Álvarez *et al*., 2015).

Sampling

In each farm, six sweet tamarind trees and six acid tamarind trees were selected, giving a total of thirty trees of each phenotypes, considered in such a way, because the characters that define a phenotype correspond in their great majority to the morphological description of the plant and its architecture (Álvarez, 2016). The trees of each phenotype were selected according to the criteria of each producer, corroborating the taste of the fruit. These were distributed in paddocks and their ages varied from 20 to 70 years of age.

A detailed revision of each tree was made *in situ,* as Nicholls (2008) suggests, from September to October 2015 and from January to February 2016 each 15 days. In addition, a sampling of the soil was made consisting in taking five subsamples from holes 20 cm deep distributed in the diameter of the area over the ground below the tree canopy, these samples were taken with a shovel.

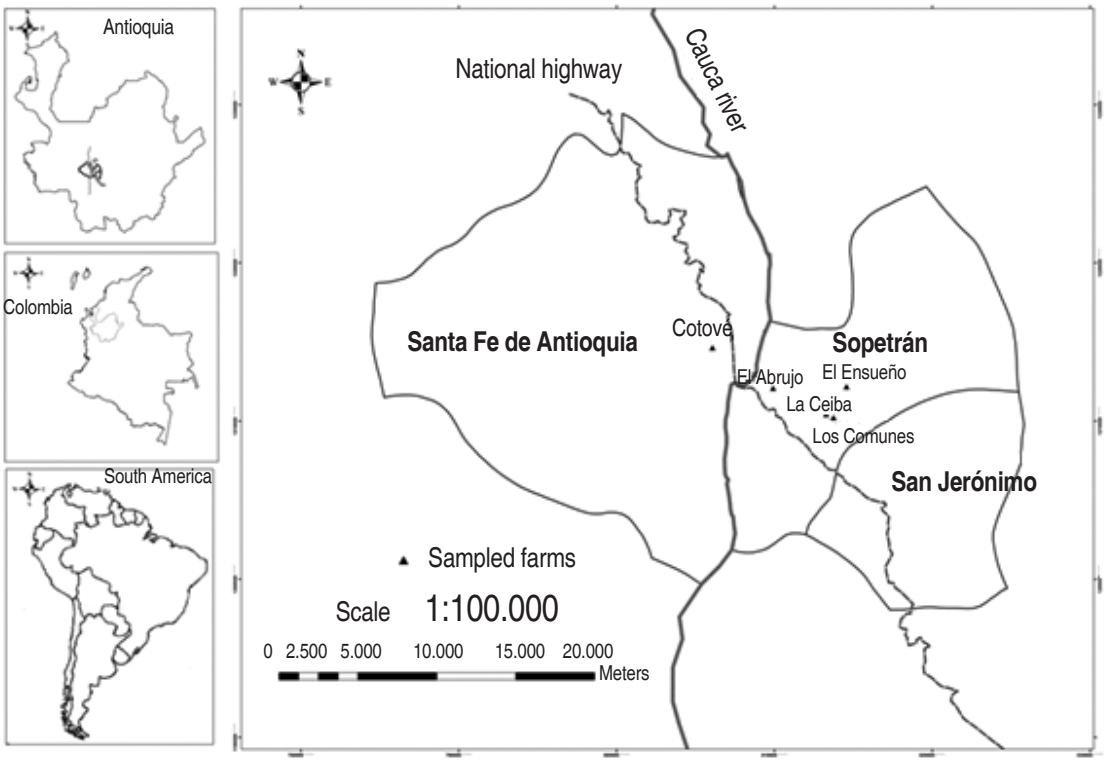

Figure 1. Geographic location of *Tamarindus indica* sampling sites, in Antioquia-Colombia.

The leaves and flowers that showed some symptom of damage were cut and stored in zip-ploc plastic bags, and the collection of insects from the symptom was done with a jama or wet brush. The adult insects were deposited in Falcon and Eppendorf tubes with 70% of alcohol with its data collection, to be identified later. The bark was revised in a similar way as the previous organs, from the base of the stem up to 1.8 m of high. In addition, a sampling of the soil around the tree was made, from which five samples were taken from under the diameter of the canopy of the tree, at a depth of 20 cm. The immature stages of insects found in all the organs were taken to the laboratory of General Botany and Plant Physiology of the Politécnico Colombiano Jaime Isaza Cadavid, in plastic boxes with food for their breeding until their adult emergence.

The evaluation of the fruit insects was done by taking 30 ripe fruits at random, obtained from those that fell to the ground after shaking the branches of the tree, discarding the old or mummified fruits. No stratification of the trees were taken into account, because the harvest of the tamarind is traditionally done in the region with the shaking of the tree method. The fruit were taken to the laboratory for reviewing. The insects in juvenile stages, present in the fruits, were grown in plastic boxes until the emergence of adults. Ten individuals of each species were conserved to describe the damage and habits, as well as to observe their possible reproduction and survival. The conditions of the laboratory were of an average temperature of 23.5 °C and a relative humidity average of 52.7%. Four individuals per species were prepared to be identified. The preparation of the Coccoidea and aphids for identification consisted of a clarification with 10% KOH, distilled water, alcohol with different concentrations, xylol, Congo red stain, clove oil and assembled with Canadian balsam (Holman, 1974). The larvae of the moths were bred and identified following the key for Pyraloidea larvae (Solis, 2006). The Insects were deposited in the Entomological Collection of the Entomological Museum Francisco Luis Gallego at La Universidad Nacional de Colombia,with codes MEFLG NC 43608, NC 43609, NC 43610, NC 43611, NC 43612, NC 43613, NC 43614, NC 43615, NC 43616, NC 43617, NC 43618, NC 43619, NC

43620, NC 43621, NC 43622, NC 43623, NC 43624, NC 43625, NC 43626, NC 43627, NC 43628, NC 43629, NC 43630, NC 43631, NC 43632, NC 43633.

Determination of the insect infestation percentage (IP)

In order to determine the IP of the insect species collected from tamarind fruits, were examined using a stereoscope NIKON®. The infestation due to each of the most important insects (*C. serratus*, *S. linearis* and the Phycitinae complex) was quantified, and then, the IP of the three together, because some fruit were simultaneously affected by several species.

The IP was calculated following the method of Montes *et al*. (2012), Montoya-Restrepo (1999), Ripa and Larral (2008) and Suárez *et al*. (2005), using the equation:

$$\%IP = \frac{Number\ of\ affected\ fruits}{Number\ of\ evaluated\ fruits} * 100$$

Determination of the grade of insect damage

For the analysis of the degree of damage a rating scale of damage was established, obtained from the observation of the harvested fruits, this consisted of ranges of damage from 0 to 5 (0, a healthy organ and 5, an organ with the highest degree of damage) and a graphic scale of these were elaborated according to the affected area in the fruits. Subsequently, a photographic record of each fruit was made, and based on this, the percentage of affected area was determined, using the free access software ImageJ®.

The graphic and descriptive scales of the degree of damage of *C. serratus* and the Phycitinae complex were made in the pulp (mesocarp and endocarp), which corresponds to the commercial part of the fruit. The scales for *S. linearis* were made with reference to the seed, since it is there where it does the most damage.

RESULTS AND DISCUSSION

Eleven (11) insects affecting tamarind trees in the region were found. The part of the plant with the highest number of associated phytophagous insects was the fruit, on the contrary, the stem and leaves had the lowest number of species (Table 1). Only the roots were unaffected.

Table 1. Taxonomic identification of phytophagous insects of *Tamarindus indica* trees in the municipalities of Sopetran and Santa Fe de Antioquia.

Order	Family	Genus	Species	Organ
Coleoptera	Bruchidae	*Caryedon*	*Caryedon serratus*(Olivier, 1790)	Fruit and seed
Coleoptera	Curculionidae	*Sitophilus*	*Sitophilus linearis* (Herbst, 1797)	Seed and fruit
Coleoptera	Curculionidae: Scolytinae	*Hypothenemus*	*Hypothenemus obscurus* (Wood and Bright, 1992)	Fruit and seed
Lepidoptera	Pyralidae: Phycitinae	____	Morph 1	Pulp
Lepidoptera	Pyralidae: Phycitinae	*Ectomyelois*	*Ectomyelois ceratoniae* (Zeller, 1839)	Pulp
Hemiptera	Aphididae	*Toxoptera*	*Toxoptera aurantii* (Boyer de Fonscolombe, 1841)	Flowers
Hemiptera	Coccoidea: Diaspididae	*Selenaspidus*	*Selenaspidus articulatus* (Morgan, 1889)	Leaves
Hymenoptera	Apidae	*Trigona*	*Trigona* sp.	Flowers
Hymenoptera	Formicidae	*Acromyrmex*	*Acromyrmex octospinosu*s (Reich, 1793)	Flowers
Hymenoptera	Formicidae	*Crematogaster* sp.	Ant associated to aphids	Flowers
Isoptera	Termitidae	*Ruptitermes*	*Ruptitermes* sp.(s.c.)	Stem

Source: Catalogue of Life (2016), Termite Database (2016).

Insects causing tamarind fruit damage

Caryedon serratus (Coleoptera: Bruchidae): Are females which deposit eggs on the epicarp of the green pod or inside of it when pods are mature; an egg is 1 mm long, approximately. Once the larva emerges, it penetrates the fruit through the pulp and when it reaches the seed,

it starts to consume it causing the main damage to this organ (Figure 2A). Furthermore, larval excretions and waste of the seed consumption contaminate the pulp. The larva may become pupa while it is in the seed, in the pulp or outside the fruit. Pupa is 7.5 mm long, approximately. Finally, the adult emerges and its size is around 7.2 mm.

Morph 1 (Lepidoptera: Pyralidae: Phycitinae): this moth was found depositing eggs on the fruit cover. The eggs are less than 1 mm long. Once the larva emerges, it penetrates the fruit and consumes the pulp while leaving its excretions there, causing the greatest damage to the organ (Figure 2B). Then, the larva becomes pupa on the pulp surface and finally, the adult emerges. Adult moth is 9.2 mm long, approximately.

Sitophilus linearis (Coleoptera: Curculionidae): The life cycle of this weevil occurs inside the seed, where it causes the greatest damage (Figure 2C). The adult has an approximated size of 4.8 mm, it drills the epicarp and the pulp and finally it reaches the seed. And there it starts to consume the endosperm, digging cavities to deposit its eggs. The larva remains at the same site and consumes the seed, where it becomes pupa. The pupa measures 4 mm long. The waste of the consumption in the different stages of the insect cause a little contamination of the pulp.

Ectomyelois ceratoniae (Lepidoptera: Pyralidae: Phycitinae): The larva affects the fruit similarly to morph species 1, however it is bigger than the morph species. *E. ceratoniae* can be found on the same fruit with morph 1 and it is difficult to differentiate each other with the bare eye.

Hypothenemus obscurus (Coleoptera: Curculionidae: Scolytinae): the adult one is 1.2 mm long. It was found drilling the epicarp (Figure 2D). While passing through the pulp, it leaves its feces and when it reaches the seed, it drills and makes tunnels inside of it.

Percentage in Insect infestation (IP)

The IP of the affected fruit by the three insects, according to the phenotype, was of 28% in the sweet phenotype, and of 21%. In the acid phenotype. *C. serratus* presented an IP of 17% and of 22%, in acid and sweet tamarind,

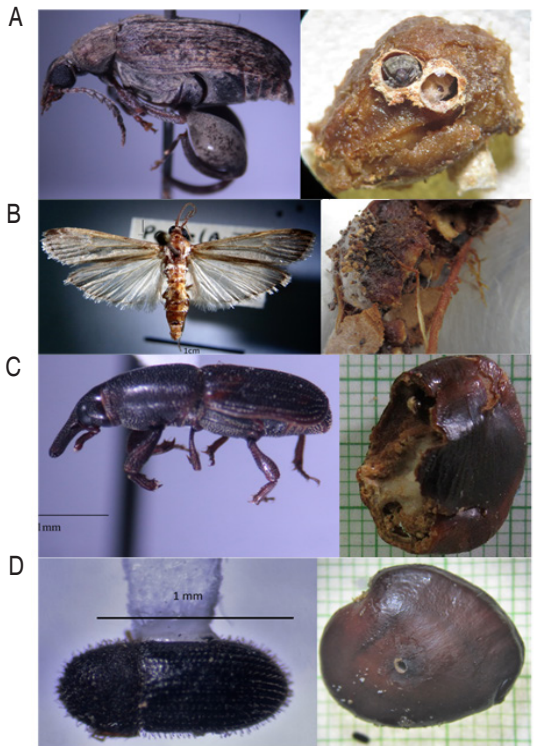

Figure 2. Phytophagous insects with their respective damage to *Tamarindus indica* L. fruit; A. *Caryedon serratus*; B. Phycitinae complex; C. *Sitophilus linearis*; D. *Hypothenemus obscurus*.

respectively. *S. linearis* recorded an IP of 2 and 3%, in acid andsweettamarind,respectively.WhilethePhycitinaemoths complex showed an IP of 6% for the acid Tamarind and 10% for the sweet tamarind. The average insect IP was 19.5%, 8% and, 2.5% for *C. serratus*, Phycitinae moths and, *S. linearis*, respectively (Figure 3).

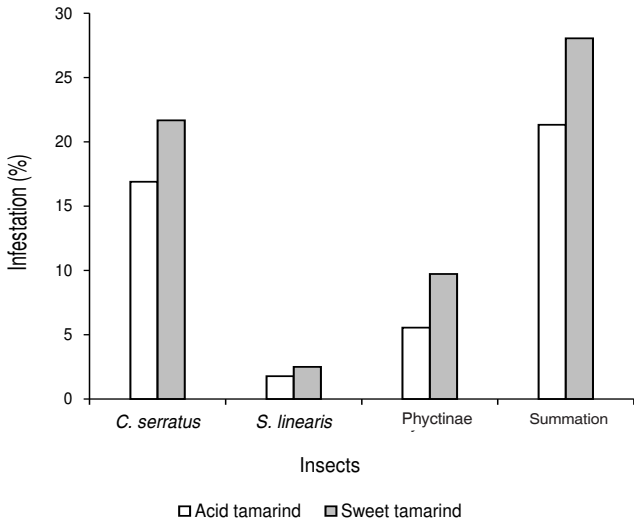

Figure 3. Percentage of Infestation (IP) of three main insects causing fruit damages to the *Tamarindus indica phenotype*.

Damage grade

The descriptive and graphic scales for *C. serratus*, the Phycitinae complex and, *S. linearis* are presented on tables 2, 3 and 4, respectively.

Table 2. Damage grade of *Caryedon serratus* in a fruit of *Tamarindus indica* L.

Grade	Damage percentage (%)	Description of the damage	Descriptive scale
0	0	Insect damage-free pulp	
1	5	Presence of larvae and feces in the pulp	
2	10	Presence of larvae, feces and, perforations in the seed	
3	20	Presence of larvae, pupae, feces and, perforations in the seed	
4	50	Presence of larvae, pupae, adults, feces and, perforations in the seed	
5	70	Presence of larvae, pupae, adults, feces and, perforations in the seed	

The highest percentage of affected fruit by *C. serratus* (43% - 52%) was recorded as grade 1 while the lowest percentage of damaged fruit by this pest (0% - 1%) was graded 5. The sweet tamarind showed more affected fruits by *C. serratus* in different damaged grades (Figure 4).

The highest percentage of tamarind fruit affected by *S. linearis* (4% - 12%) was registered as grade 1 while the other grades were found in a very low percentage of affected fruits (0% - 1%) by this insect. the acid tamarind fruit was classified in grade 1 (12%), on the other hand,

Table 3. Degree of damage of Phycitinae complex in a fruit of *Tamarindus indica* L.

Grade	Damage percentage (%)	Description of the damage	Descriptive scale
0	0	Insect damage-free pulp	
1	10	Presence of larvae and/or feces	
2	28	Presence of larvae and/or feces	
3	45	Presence of larvae, feces and, silk web	
4	65	Presence of larvae, pupa, feces and, silk web	
5	90	Presence of larvae, pupa, adults, feces and, silk web	

Table 4. Degree of damage by *Sitophilus linearis* in the seed of *Tamarindus indica* L.

Grade	Damage percentage (%)	Description of the damage	Descriptive scale
0	0	Insect damage-free seed	
1	7	Adults and, bored seed	
2	11	Adults, eggs and, bored seed	
3	20	Adults, eggs, larvae and, bored seed	
4	40	Adults, eggs, larvae, pupae and, bored seed	
5	55	Adults, eggs, larvae, pupae and, bored seed	

the classification of the sweet tamarind fruit damaged by *S. linearis* corresponded to grades 1, 2, 3 and, 5.

The highest percentage of fruit affected by the moths of Phycitinae (6% - 9%) had grade 1, and the lowest percentage (3% - 4%) was classified in grade 4. The tamarind acid fruit also presented the highest percentage of insect damage (9%) that had grade 1 and, only 8% of tamarind sweet fruit damaged by this pest was recorded with grade 2. The average percentage of tamarind affected fruits having in the different grades of damage (5.6%) occurred in those from sweet tamarind phenotype.

The number of species found in this study (11) does not overcome those reported in tamarind in Mexico (14) (Orozco, 2001). In the present work, five new record were recorded for *T. Indica* in Colombia: *Ectomyelois ceratoniae, Hypothenemus obscurus*, *Toxoptera aurantii, Trigona* sp. and, *Acromyrmex octospinosus*. In both Mexico and Colombia, *C. serratus*, *S. linearis* and, the *Cadra* genus (syn. *Ephestia)* (Orozco *et al*., 2009; Muñoz and Rueda, 2009) are the most important species affecting tamarind fruit. Actually differing from those registered in other regions of the world (Gupta *et al*., 2017). This reveals the importance of these coleopteran species as key pest

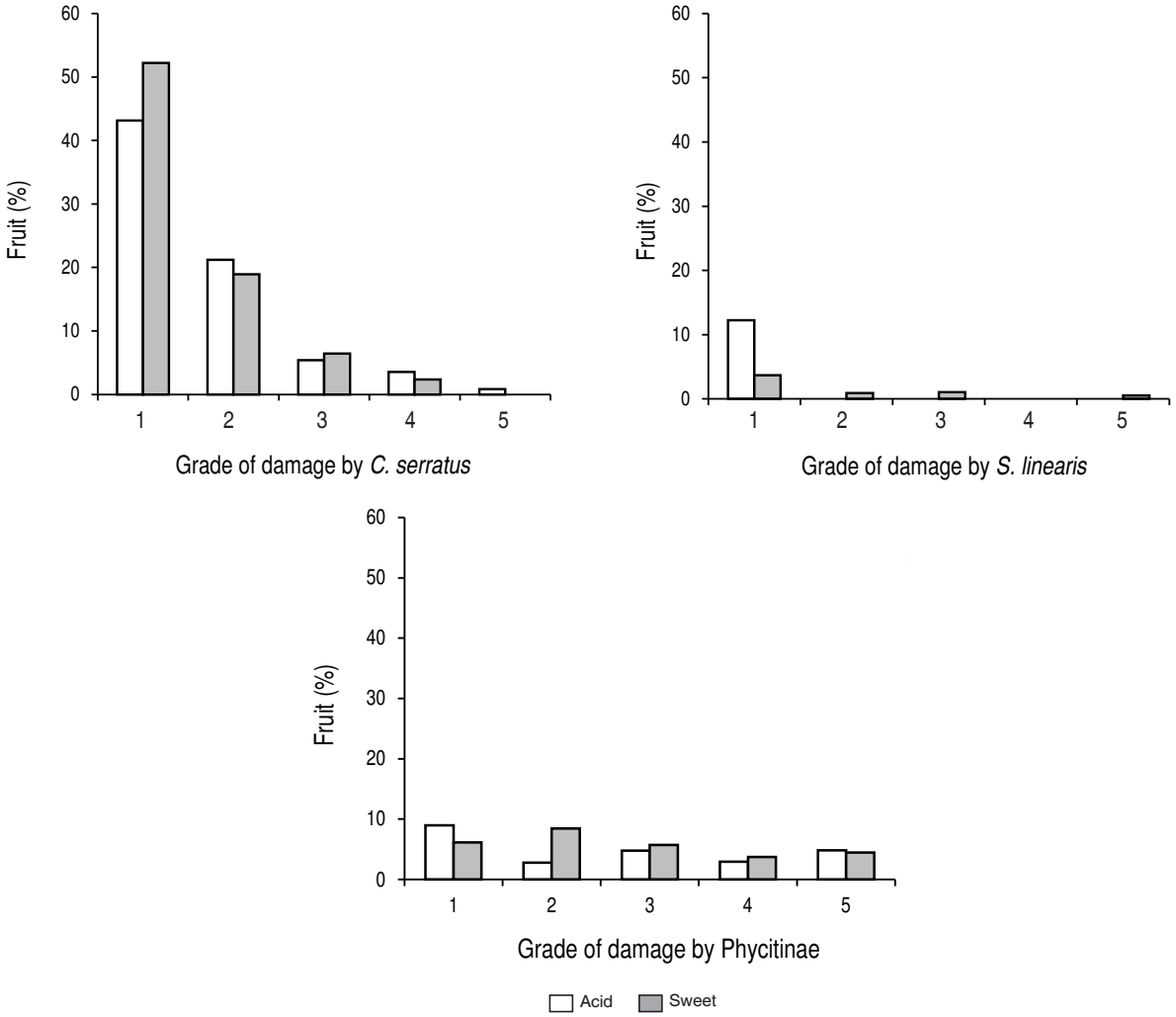

Figure 4. Percentage of affected *Tamarindus indica* L. fruit by three insect pests.

in the neotropical tamarind areas. In addition, in our study, we found two new Lepidoptera species previously unreported in the country.

Caryedon serratus was registered for the first time in tamarind crop in the region where this study was done (Vélez, 1972), in the municipality of Santa Marta (Magdalena department – Colombia). So the continuity of this specie is conformed as an important tamarind insect pest.

Sitophilus linearis was reported in the United States by Cotton (1920), in Mexico by Orozco *et al.* (2009) and, by Muñoz and Rueda (2009) in the studied region. In this study, it was proved that this insect affects the seed. However, before the seed, the adult passes through the pulp affecting its quality.

The complex of Phycitinae moths reported in this study are different from those moths reported by Muñoz and Rueda (2009) and Muñoz *et al.* (2014) in the same Colombian region (*C. cautella* and *Amyelois transitella*). Thus, it is evident that various Lepidoptera species are pests of tamarind fruit in the nearby western of Antioquia. While the Coleoptera species *H. obscurus* affects the seed, documented by Wood (2007) affecting the tamarind crop as one of various hosts.

Sweet tamarind may be more susceptible to insect infestation, probably because the trees have very little

or none management at all. Moreover, some fruit are not harvested remaining on the trees, stimulating the insect permanence, hence its life cycle and reproduction is not interrupted. This difference in crop management is due to the limited commercialization of sweet tamarind, because the acid one is preferred for making confections that needs sugar.

CONCLUSIONS

In this study, the most important tamarind insect pests with the highest IP were *C. serratus* (17-22%), the Phycitinae moths (6%-10%) and, *S. linearis* (2%-3%). In general, the most affected fruits had more than one insect pest, and the degree of affectation was low. Studies on the abundances of the phytophagous insects of the fruit, should be carried out to quantify aspects such as the effect on the phenotype (acid, and sweet tamarind), the management carried out by the producer and the age of the trees, and the insects of the fruit. It is important to evaluate some cultivation practices, and the use of low impact biological insecticides, can contribute to improve the products derived from this fruit. It is also necessary to evaluate the economic thresholds and levels of economic damage, to determine the effect of these insects on the production.

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Research article

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Antifungal activity of extracts, essential oil and constituents from *Petroselinum crispum* against *Colletotrichum acutatum*

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Actividad antifúngica de extractos, aceite esencial y constituyentes de *Petroselinum crispum* contra *Colletotrichum acutatum*

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ABSTRACT

Keywords:
Apiaceae
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GC-MS
Parsley-apiole

The effect of extracts, essential oil, and their major constituents from parsley (*Petroselinum crispum* (Mill.) Fuss.) against the phytopathogenic fungus *Collectotrichum acutatum* was evaluated by the poisoned agar method. Results showed that all extracts, along with the essential oil, significantly inhibit the radial growth of *C. acutatum* at concentrations higher than 100 µg mL^{-1}. The higher activity was found for the essential oil followed by the *n*-hexane extract. Analysis by gas chromatography with mass spectroscopy (GC-MS) of *n*-hexane extract and the essential oil of *P. crispum* showed that the major components correspond to the phenylpropanoids myristicin and *parsley*-apiole. Both compounds were isolated by conventional chromatographic techniques and their structures elucidated by spectroscopic methods. Myristicin and *parsley*-apiole displayed a significant inhibitory effect against *C. acutatum*. The highest fungistatic activity was found to *parsley*-apiole with IC_{50} value of 40 µg mL^{-1}. In conclusion, parsley may be a good source of antifungal compounds to control *C. acutatum*.

RESUMEN

Palabras clave:
Apiaceae
Perejil
Extractos
Aceite esencial
CG-EM
Parsley-apiol

Se evaluó el efecto de extractos, el aceite esencial y los componentes principales del perejil (*Petroselinum crispum* (Mill.) Fuss.) contra el hongo fitopatógeno *Collectotrichum acutatum* mediante el método del agar envenenado. Los resultados mostraron que todos los extractos, junto con el aceite esencial, inhibieron significativamente el crecimiento radial de *C. acutatum* en concentraciones superiores a 100 µg mL^{-1}. La mayor actividad se encontró para el aceite esencial, seguido por el extracto de *n*-hexano. El análisis por cromatografía de gases con espectroscopia de masas (CG-EM) del extracto de *n*-hexano y el aceite esencial de *P. crispum* mostró que los componentes principales corresponden a los fenilpropanoides miristicina y *parsley*-apiol. Ambos compuestos fueron aislaron por técnicas cromatográficas convencionales y sus estructuras elucidadas por métodos espectroscópicos. La miristicina y el *parsley*-apiol mostraron un efecto inhibidor significativo contra *C. acutatum*. La actividad fungistática más alta se encontró para *parsley*-apiol con un valor CI_{50} de 40 µg mL^{-1}. En conclusión, el perejil puede ser una buena fuente de compuestos antimicóticos para controlar el *C. acutatum*.

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Anthracnose, a disease caused by the phytopathogenic fungus *Colletotrichum acutatum* Simmonds affects different plants, such as tamarillo (*Solanum betaceum* Cav.), tomato (*Solanum lycopersicum* L.), strawberry (*Fragaria x ananassa* Duch.), apple (*Malus domestica* Borkh.), blackberry (*Rubus glaucus* Benth.), mango (*Mangifera indica* L.), among other fruits of economic importance (Damm *et al*., 2012; Wharton and Diéguez-Uribeondo, 2004). Its presence occurs in temperate and tropical climates around the world. Conventionally, its control has been based on the application of synthetic fungicides, which can cause adverse effects to the environment and to man because of their low selectivity (Gao *et al*., 2017; Wharton and Diéguez-Uribeondo, 2004; Gaviria-Hernández *et al*., 2013). In addition, as a result of the gradual development of resistance by the microorganism to this class of substances used for its control, it has become necessary to apply higher and more constant doses in the field, which has led to an increase in the production costs and furthers the problem of food security (Jílková *et al*., 2015**)**.

In response to the above, new alternatives of control disease in important crops are needed. One of the approaches that have become more prevelant in recent years is the one that uses essential oils and extracts of plants or their major components for the control of phytopathogenic microorganisms (Grande-Tovar *et al*., 2018; Nazzaro *et al*., 2017). Alternative methods to control *C. acutatum* have been developed; these include, the use of natural antimicrobials, biocontrol agents, ultraviolet radiation and resistant cultivars (Falconí and Yánez-Mendizábal, 2018). For example, He *et al*. (2018) determined the efficacy and possible mechanism of cinnamon essential oil on inhibition of *C. acutatum* isolated from kiwi fruit. Meanwhile, Rashid *et al*. (2018) evaluated the antifungal effects of *Rhus coriaria* L. fruit extracts against tomato anthracnose caused by *Colletotrichum acutatum*. The results indicate that *R. coriaria* extract can be utilised as a deterrent and curative natural product for the anthracnose disease in tomatoes. Some substances of this type have demonstrated a high antimicrobial activity, good selectivity, low toxicity, and little persistence in the environment (Tripathi and Dubey, 2004).

Edible and herbal plants/spices such as garlic, oregano, cinnamon, clove, parsley, among other are an important source of essential oils and have been employed alone or combined as natural food preservatives with antimicrobial properties (Pisoschi *et al*., 2018). Particularly, parsley is an easy plant to grow with low production costs, and is an aromatic herb commonly used in Colombia. The essential oil and different extracts obtained from parsley have shown antimicrobial activity against Gram positive and negative bacteria (Khalil *et al*., 2018; Semeniuc *et al*., 2017; Farzaei *et al*., 2013). To the extent of our knowledge, there are few works to establish the antifungal potential of compounds obtained from parsley. In the present work, the antifungal activity against *C. acutatum* of *n*-hexane, dichloromethane, ethyl acetate, and methanol extracts, the essential oil, and their main constituents from parsley was evaluated.

MATERIALS AND METHODS

Chemicals

NaOH, H_2SO_4, HCl, Na_2SO_4, glucose, K_2HPO_4, $NaNO_3$, $MgSO_4$ $FeSO_4$ were purchased from Merck KGaA (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO, USA). Solvents methanol (MeOH), dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), and *n*-hexane were acquired from Merck. All chemicals and reagents used were of analytical grade.

General methods

Thin layer chromatography was performed with silica gel F_{254} (0.2 mm Merck) plates. Compounds were detected by UV fluorescence (254 and 365 nm) and/or spraying with AcOH-H_2SO_4-H_2O (143:28:30, v/v) "Universal stain", followed by heating at 80 °C for 1–2 min. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AMX 300 spectrometer (^1H at 300 MHz; ^{13}C 75.5 MHz). Chemical shifts (δ) are reported in ppm relative to the residual solvent signals ($CDCl_3$: δ_H 7.24 and δ_C 77 ppm) and coupling constants (*J*) in Hz. Mass spectrometry analysis was performed using a Hewlett-Packard 6890 (Agilent Technologies) gas chromatograph coupled with a HP 5973 MSD (Mass selective detector-Quadrupole type). A HP-5 MS capillary column (30 m x 0.25 mm x 0.25 µm, Agilent Technologies) was used. Hydrogen was the carrier gas, having a constant flow rate of 1.0 mL min^{-1} and split ratio 1:10. The temperature was programmed as follows: column temperature, 50 °C; injector temperature, 150 °C; detector temperature, 280 °C. The oven temperature was programmed from 50 to 250 °C at 10 °C min^{-1} and, then, held isothermally

for 6 min. The MS system operated with an electron impact of 70 eV, an acceleration voltage of 1.1 kV, and scan range of *m/z* 40-500. The products were identified by comparison of their mass spectra with those of the US National Institute of Standards and Technology (NIST) mass spectral library (Version 2008). Quantitative analysis of each essential oil component (expressed in percentages) was carried out by peak area normalization measurement.

Plant material and extraction procedure

Extracts: Fresh parsley plant materials (curly leaf) were purchased from a local supermarket in Medellín, Colombia. Samples were washed under tap water and roots were discarded. Leaves and stems were homogenized in a laboratory blender. Then, vegetal material (900 g) was extracted sequentially with 700 mL of each solvent (*n*-hexane (HEX), dichloromethane (DCM), ethyl acetate (EA), and methanol (ME)) by percolation (5 cycles per 24 h) a room temperature until exhaustion. Extracts were dried over anhydrous sodium sulfate, filtered, evaporated using a rotary evaporator (Buchi Heating Bath B-490, Buchi Rotavapor R-200) under reduced pressure at 40 °C, and stored in a refrigerator at 4 °C in amber vials. *Essential oil*: The essential oil (EO) was obtained by hydrodistillation. A quantity of 900 g of fresh leaves and stems of parsley was coarsely chopped and immersed in 1.5 L of distilled water contained in a 3 L flask. Distillation was carried out using a modified Clevenger apparatus. The extraction process was performed for 2 h after the first drop of distillate until complete exhaustion of the plant. Then, sodium chloride (1 g) and 40 mL of dichloromethane (2x20 mL) was added with the aqueous distillate in a separating funnel. The organic layer was separated, dried over sodium sulfate, filtered, evaporated using a rotary evaporator (Buchi Heating Bath B-490, Buchi Rotavapor R-200) under reduced pressure at 40 °C, and stored in a refrigerator at 4 °C in amber vials.

Isolation and identification of major constituents in bioactive materials

The bioactive *n*-hexane extract and essential oil from *P. crispum* were measured chromatographically with silica gel (Merck, Darmstadt, Germany, silica gel 60, 230-400 mesh) column and eluted with gradients of *n*-hexane:EtOAc (100:0, 95:5, 90:10, 80:20, 70:30, 0:100 v/v). Next, the *n*-hexane and *n*-hexane:EtOAc (95:5) fractions were measured in a silica gel column chromatograph eluted with *n*-hexane and gradients *n*-hexane:CH_2Cl_2 (98:2, 95:5, and 90:10 v/v). Fractions 2 to 4 were mixed and measured through a Sephadex LH-20 column chromatograph eluted with *n*-hexane:CH_2Cl_2:MeOH, 50:25:25, v/v affording two compounds, which were identified through spectroscopic methods (one-dimensional (^{1}H and ^{13}C) and two-dimensional (COSY, HSQC and HMBC) NMR, as well as EI/MS analysis.

Antifungal activity

The strain of phytopathogenic fungus *C. acutatum* was isolated from commercial fruits of tamarillo (*S. betaceum*) affected by the disease and purchased at the local markets in the city of Medellín (Colombia). Identification was performed by the Phytopathology Laboratory of the Universidad Nacional de Colombia-Sede Medellín, evaluating morphological and molecular characteristics (Afanador-Kafuri *et al*., 2014). The evaluation of antifungal activity was carried out using the poisoned agar method (Balouiri *et al*., 2016). Essential oil, extracts, and pure compounds were mixed with ethanol (<0.2% EtOH) and tested at 100, 200, and 400 µg mL^{-1}. Thymol (2-isopropyl-5-methylphenol) and carbendazim (methyl benzimidazol-2-ylcarbamate) at 100 µg mL^{-1} were used as positive controls. Mycelial growth (mm) were determined as mean values ± standard deviation of three replicates of mycelium diameter. In addition, for bioactive materials and their major constituents, the concentration that inhibits 50% fungal mycelia growth (IC_{50}) was determined according to Rivillas and Soriano (2007).

RESULTS AND DISCUSSION

Table 1 shows the extraction yield for the four solvents of different polarities –*n*-hexane (HEX), dichloromethane (DCM), ethyl acetate (EA), and methanol (ME)-, and the essential oil (EO) collected by hydrodistillation from *P. crispum*. The order of increasing yield was HEX < DCM < EA < ME, coinciding with a growing polarity of the extracting solvent. Thus, the highest yield was achieved by the most polar solvent, methanol. The *n*-hexane extract presented approximately 10 times less of the weight reached by the methanol extract. This suggests that the proportion of low polarity products extractable with hexane in *P. crispum* is low, compared to those of high polarity present in extracts in ethyl acetate and methanol. The yield from essential oil hydrodestillation

extraction was closer to the extraction yield from *n*-hexane. Overall, the total amount of extractable material with organic solvent in *P. crispum* was 13.73 g (1.42%).

Then, all extracts and essential oil were tested for antifungal activity using the poisoned-food technique at 100, 200, and 400 µg mL^{-1}, according to Balouiri *et al.* (2016).

Table 1. Yields of essential oil and extracts for the leaves and stems of *P. crispum.*

Extract	Mass (g)[a]	%(p/p)[b]
HEX extract	0.72	0.08
DCM extract	1.67	0.19
EA extract	2.03	0.22
ME extract	8.32	0.92
Essential oil	0.99	0.11

[a] Crude weight results from 900 g of fresh aerial parts. [b] Yield (%) = [crude weight/900 g]×100

The inhibitory effects of organic extracts and essential oil of *P. crispum* at 400 µg mL^{-1} on the growth of *C. acutatum* are presented in Figure 1. All extracts displayed a significant inhibitory effect in the growth of *C. acutatum* after 264 h. In general, the growth of fungal mycelium was dependent of the type and amount of extract in the culture medium. The *n*-hexane extract caused the least growth of *C. acutatum*; after 264 hours, the radial growth was almost 30 mm less than the control. Higher polarity extracts, methanol followed by ethyl acetate, had the lowest inhibitory effect on *C. acutatum*. After 72 h, the *n*-hexane extract showed an inhibition percentage of 57%. Then, the fungistatic effect presented a slight decrease, reaching 48% inhibition after 264 h.

The essential oil of *P. crispum* displayed the highest inhibitory effect. At 400 µg mL^{-1}, essential oil of *P. crispum* inhibited the radial growth of *C. acutatum*

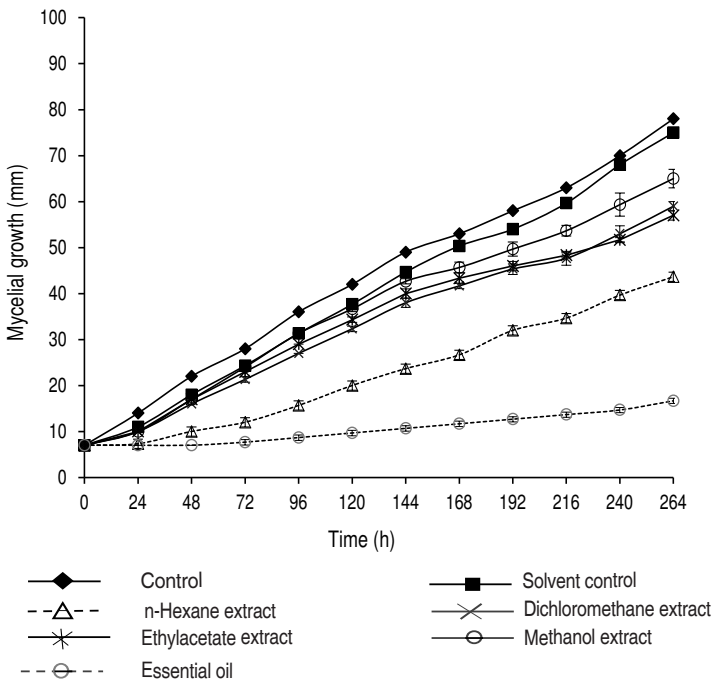


Figure 1. Effect of extracts and essential oil of *Petroselinum crispum* at 400 µg mL^{-1} on radial mycelial growth of *Colletotrichum acutatum.*

completely during the first 72 h. Similarly, the inhibition percentage was slightly decreased at 78% after 264 h. Even at 100 µg mL^{-1}, essential oil exhibited inhibition percentages of *C. acutatum* of almost 60% during the 264 h of the analysis (*data not shown*). Thymol and carbendazim® presented a significantly higher antifungal effect (inhibition percentages of 100%) as compared to all evaluated extracts. Carbendazim is commonly used for control of anthracnose disease, both in the field and postharvest. However, some plant pathogens, such as *C. acutatum* and *C. gloeosporioides,* have strains resistant to carbendazim (Zhang and Huang, 2007). Meanwhile, thymol has been recognized for its potent antifungal activity (Numpaque *et al*., 2011). The IC_{50} values calculated for the *n*-hexane extract and the essential oil of *P. crispum* were 316.7 and 185.1 µg mL^{-1}, respectively. The high antifungal activity of essential oil of *P. crispum* found in the present study is in agreement with that reported by Linde *et al*. (2016). The chemical composition of essential oil and *n*-hexane extract from leaves and stems of *P. crispum* was analyzed by means of GC-MS. The detected constituents (relative amounts higher than 0.1%) are shown in Tables 2 and 3. Analysis by GC-MS led to the identification and quantification of about 23 components. Among them were 12 in the essential oil and 22 in the *n*-hexane extract, comprising between 93.5-99.1% of the total composition of the essential oil/*n*-hexane extract. Phenylpropenes were the main components detected in the essential oil (77.0%) and *n*-hexane extracts (48.7%) for *P. crispum*. *Parsley*- or *dill*-apiole (27.6 and 43.2% in *n*-hexane extract and essential oil, respectively) and myristicin or sarisan (18.7 and 30.8% in *n*-hexane extract and essential oil, respectively) were the major constituents on *P. crispum*. The phenylpropene safrole was also detected in low proportion.

Table 2. Relative abundance (%RA) of major compounds obtained by GC-MS (%) from *n*-hexane extract of *P. crispum*.

TR (min)	Component	%RA	Fragment (m/z)
5.57	D-Limonene	0.2	68(100), 93(59), 67(45), 79(23), 94(23), 136(23)
5.71	β-Phellandrene	8.9	93(100), 77(27), 91(25), 136(18), 79(17), 94(15)
6.87	Terpinolene	1.5	121(100), 93(98), 136(73), 79(34), 77(30), 91(31)
7.52	*p*-Cymene	5.7	117(100), 132(81), 115(63), 91(54), 92(23), 65(23)
7.76	1,3,8-*p*-Menthatriene	11.2	119(100), 91(74), 134(66), 41(30), 39(27), 105(21)
9.34	Geranial	2.1	69(100), 41(46), 84(29), 94(17), 83(14), 67(12)
10.34	Melilotal	0.8	119(100), 91(64), 134(37), 65(14), 120(10), 39(8)
10.73	Pentylideneciclohexane	0.6	67(100), 109(77), 81(60), 96(54), 41(37), 152(37)
11.19	3-Guaiacylpropanol	1.5	91(100), 121(63), 166(30), 77(25), 65(19), 51(18)
12.13	Safrole	2.4	162(100), 104(39), 131(35), 135(31), 103(27), 77(27)
13.19	β-Caryophyllene	0.6	93(100), 133(92), 91(86), 41(77), 79(76), 69(75)
14.27	2-Methylisoborneol	1.2	95(100), 107(25), 43(22), 108(21), 41(16), 110(15)
15.33	β-Sesquiphellandrene	0.9	69(100), 41(68), 93(46), 55(29), 91(28), 92(24)
15.97	Ledol	1.2	43(100), 41(61), 69(53), 109(50), 81(34), 55(34)
17.79	Myristicin or sarisan	18.7	192(100), 91(25), 165(23), 65(16), 119(15), 161(14)
21.17	*Parsley*- or *dill*-apiole	27.6	222(100), 207(24), 149(16), 177(16), 223(14), 195(11)
23.86	Hexadecanoic acid	3.8	43(100), 73(97), 60(90), 41(77), 57(77), 55(65)
24.14	Ethyl linoleate	0.6	79(100), 67(63), 95(60), 93(52), 55(46), 81(44)
24.35	Methyl linoleate	1.8	79(100), 67(78), 41(61), 55(57), 93(47), 81(43)
24.78	Phenethyl hexanoate	2.1	104(100), 105(18), 43(12), 99(6), 71(5), 91(5)
28.82	Linoleic acid	3.2	67(100), 81(88), 82(73), 95(63), 68(60), 55(60)
29.60	α-Linolenic acid	2.5	41(100), 79(78), 67(73), 55(62), 81(38), 80(37)

Monoterpenes (27.5%), fatty acids (11.9%), oxygenated monoterpenes (3.3%), sesquiterpenes (2.7%), and others (5.0%) were also found in the extract. Meanwhile, 7.5% monoterpenes, 0.6% oxygenated monoterpenes, 3.9% sesquiterpenes, and 4.5% others were detected in the essential oil.

Table 3. Relative abundance of major compounds obtained by GC-MS (%) from essential oil of *P. crispum*.

TR (min)	Component	%RA	Fragment (m/z)
8.08	D-Limonene	0.7	68(100), 93(59), 67(45), 79(23), 94(23), 136(23)
8.30	β-Phellandrene	1.6	93(100), 77(27), 91(25), 136(18), 79(17), 94(15)
8.53	*p*-Cymene	4.4	117(100), 132(81), 115(63), 91(54), 92(23), 65(23)
10.75	1,3,8-*p*-Menthatriene	0.8	119(100), 91(74), 134(66), 41(30), 39(27), 105(21)
12.87	β-Phenylethanol	0.7	91(100), 92(56), 65(23), 122(22), 39(12), 51(9)
13.36	Geranial	0.6	69(100), 41(46), 84(29), 94(17), 83(14), 67(12)
14.81	Melilotal	3.8	119(100), 91(64), 134(37), 65(14), 120(10), 39(8)
17.24	Safrole	3.0	162(100), 104(39), 131(36), 103(35), 77(27), 161(26)
18.68	β-Caryophyllene	1.1	41(100), 69(98), 93(94), 133(65), 79(61), 91(55)
22.03	Ledol	2.8	43(100), 41(61), 69(53), 109(50), 81(34), 55(34)
24.72	Myristicin or sarisan	30.8	192(100), 91(24), 165(22), 119(16), 65(15), 39(14)
29.41	*Parsley*- or *dill*-apiole	43.2	222(100), 207(24), 149(16), 177(16), 223(14), 195(11)

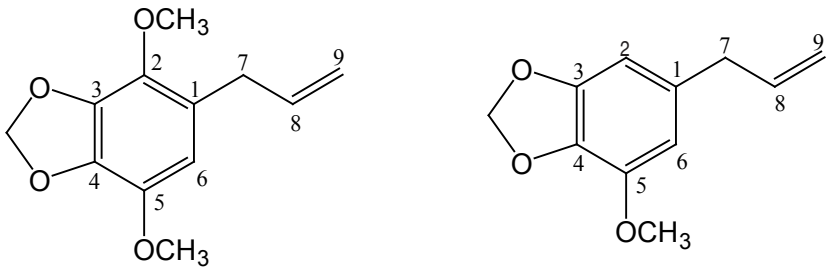
Previous reports differ on the major constituents in essential oil of curley leaf parsley. Some authors have found that monoterpenes are the dominant compounds. Thus, El-Zaeddi *et al*. (2016b) reported that 1,3,8-*p*-menthatriene (38.4-48.8%) and β-phellandrene (22.2-29.5%) are the major constituents in the essential oil of parsley grown in Spain, obtained by hydrodistillation using a Deryng system (the Polish version of the Clevenger apparatus). Kurowska and Galazka (2006) found α-pineno (32.0%) to be the major constituent of the essential oil of *P. crispum* cultivated in Poland, also having β-pineno (19.0%), myristicin (18.3%), and apiole (10.1%). On the other hand, Camilotti *et al*. (2015) reported that phenylpropanoides were the principal compounds, comprising 52.07% of the oil and consisting mainly of apiole (41.05%) and myristicin (5.08%). According to Zhang *et al*. (2006), myristicin (32.75%) and apiole (17.54%) were the major constituent in essential oil of parsley cultivated in China. Stankovic *et al*. (2004) found that apiole (57.0%) was the major constituent of essential oil of *P. crispum* cultivated in Serbia and Montenegro. Linde *et al*. (2016) reported apiole (50.3%) and myristicin (14.0%) as major constituents in the essential oil of parsley cultivated in Brazil. The chemical composition of the parsley is also dependent on the variety analyzed; in the flat-leaf parsley (*Petroselinum sativum*) contents of myristicin and apiol have been found that vary between 25-47% and 18-23%, respectively (Romeilah *et al*., 2010; El-Zaeddi *et al*., 2016a). In this way, apiole and/or myristicin have always been among the major constituents of essential oils obtained from parsley. This diverse chemical composition could be a result of several parameters including geographical origin, vegetative stage of plant, parsley cultivar, storage condition, and extraction method, as well as their interactions with microorganisms and insects (Chatzopoulou and Katsiotis, 1995). In addition, abiotic factors like luminosity, temperature, rainfall, nutrition, time of the day for harvest, and post-harvest techniques are also important to the chemical composition of essential oil (Morais and Castanha, 2012). The results by Camilotti *et al*. (2015), Zhang *et al*. (2006), Linde *et al*. (2016) and Stankovic *et al*. (2004) agree quite well with the results of this study.

Then, the *n*-hexane extract and essential oil from *P. crispum* were submitted to chromatography on silica gel, using *n*-hexane and *n*-hexane:EtOAc (95:5). Further

sequential purification by column chromatography using silica gel (eluted with *n*-hexane and *n*-hexane:CH_2Cl_2, 98:2) and Sephadex LH-20 (eluted with *n*-hexane:CH_2Cl_2:MeOH, 50:25:25) , which were identified through one-dimensional (^1H and ^{13}C) and two-dimensional (COSY, HSQC, and HMBC) NMR spectroscopic methods, in addition to EI/MS analysis.

Parsley-apiole: The compound was isolated as a yellowish oil; $C_{12}H_{14}O_4$. EI-MS m/z: 222(100)$[M]^+$, 207 (26), 195 (11), 191 (11), 177 (18), 149 (16). ^1H NMR (300 MHz, $CDCl_3$): δ 6.32 (1H, *s*, H-2), 5.95 (2H, *s*, -OCH_2O-), 5.90-6.00 (1H, *m*, H-8), 5.08 (1H, *dd*, *J* = 1.5, 12.9, H-9b), 5.03 (1H, *dd*, *J* = 1.5, 9.6, H-9a), 3.89 (3H, *s*, -OCH_3), 3.86 (3H, *s*, -OCH_3), 3.31 (2H, *dd*, *J* = 1.5, 6.3, H-7). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 138.9 (C-3), 138.6 (C-5), 137.2 (C-8), 136.1 (C-2), 135.0 (C-4), 125.6 (C-9), 115.1 (C-1), 108.1 (C-6), 101.3 (-OCH_2O-), 59.9 (-OCH_3), 56.7 (-OCH_3), 33.9 (C-7).

Myristicin: The compound was isolated as a colorless oil; $C_{11}H_{12}O_3$. EI-MS m/z: 192(100)$[M]^+$, 165 (22), 161 (13), 119 (16), 91 (24). ^1H NMR (300 MHz, $CDCl_3$): δ 6.29 (1H, *d*, *J* = 1.4, H-2), 6.26 (1H, *d*, *J* = 1.4, H-6), 5.83 (2H, *s*, -OCH_2O-), 5.77 (1H, *m*, H-8), 5.03 (1H, *dd*, *J* = 1.5, 17.0, H-9a), 4.92 (1H, *dd*, *J* = 1.5, 8.1, H-9b), 3.79 (3H, *s*, -OCH_3), 3.21 (2H, *d*, *J* = 6.3, H-7). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 144.9 (C-4), 143.6 (C-5), 137.4 (C-3), 136.7 (C-8), 125.6 (C-1), 115.9 (C-9), 107.6 (C-2), 102.7 (C-6), 101.3 (-OCH_2O-), 50.9 (-OCH_3), 33.3 (C-7). Spectral analyses were in agreement with those reported in the literature (Di Stefano *et al*., 2011; Razzaghi-Abyaneh *et al*., 2007). Chemical structures are presented in Figure 2.



parsley-Apiole Myristicin

Figure 2. Isolated compounds from *P. crispum*.

Antifungal activity of isolated compounds was evaluated for *C. acutatum*. Results are presented in Figures 3 and 4. The myristicin (a phenylpropanoid with one methylenedioxy and one methoxy group) presented a moderate fungistatic activity for all the evaluated concentrations, as compared to thymol and the carbamate carbendazim®. However, it was observed that even at the concentration of 100 µg mL^{-1}, myristicin presented a significant reduction of mycelial growth of the microorganism, as compared to the control and solvent control. In general, the fungistatic activity of myristicin was concentration dependent. At 200 µg mL^{-1}, the inhibition percentage was greater than 35%, even after 264 h. At this time, the concentration of 400 µg mL^{-1} showed a 55% inhibition.

The moderate antifungal property of myristicin has been previously reported (Moreira-Valente *et al*., 2015; Meepagala *et al*., 2005). According to Moreira-Valente *et al*. (2015), myristicin at 0.3% inhibited *Aspergillus flavus* and *A. ochraceus* 85 and 80%, respectively.

Parsley-Apiole (a phenylpropanoid with one methylenedioxy and two methoxy groups) had a high fungistatic activity against the phytopathogenic fungus *C. acutatum*. The development of fungal colonies was initiated only after 24 hours of inoculation at a concentration of 75 µg mL^{-1} and after 48 hours for other concentrations evaluated. Growth inhibition percentages were found to be between 100 and 65% after 96 h at concentrations greater than 100 µg mL^{-1}. The IC_{50} calculated for *parsley*-apiole was 40.0 µg mL^{-1}. In an earlier work on the antifungal properties of *parsley*-apiole, it was shown to have a varying degree of growth inhibitory effects against *B. cinerea*, *C. acutatum*, *C. fragariae*, *C. gloeosporioides,* and *F. oxysporium*

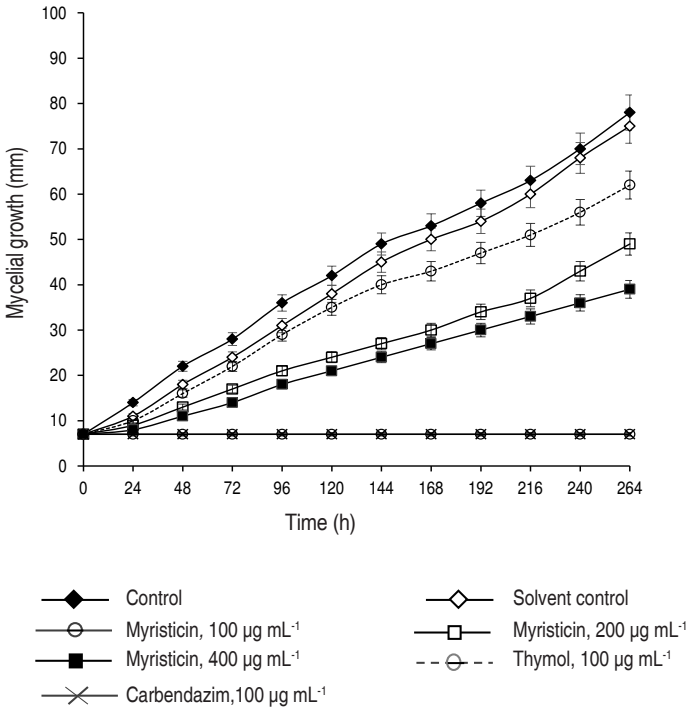


Figure 3. Effect of myristicin on radial mycelial growth of *C. acutatum*.

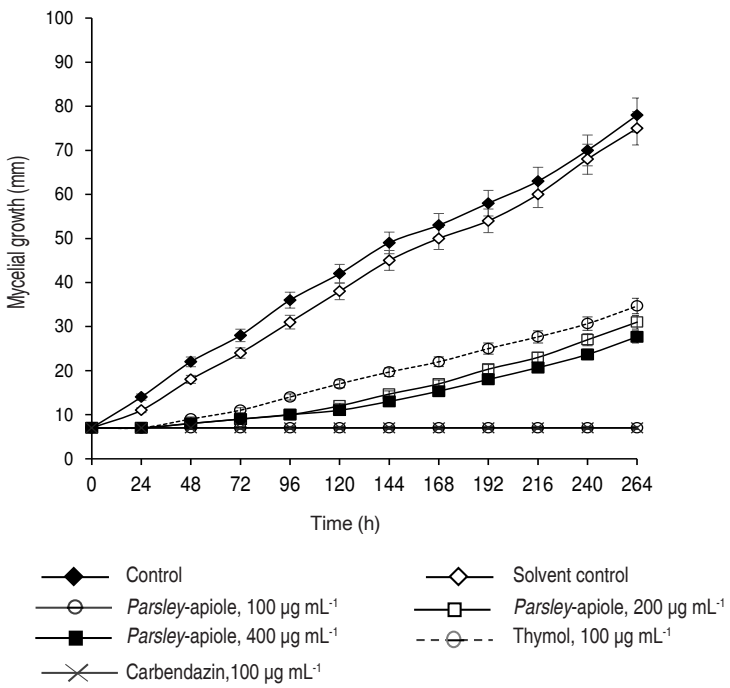


Figure 4. Effect of *parsley*-apiole on radial mycelial growth of *C. acutatum*.

(Meepagala *et al*., 2005). The authors showed that *parsley*-apiole exhibited high antifungal activity against *B. cinerea* but low activity against *C. acutatum*, *C. fragariae*, *C. gloeosporioides*, and *F. oxysporium* in the microdilution assay (Meepagala *et al*., 2005). This apparent discrepancy with respect to the present results could be attributed to the fact that different strains of *C. acutatum* and different methods were used to evaluate antifungal activity. In addition, *parsley*-apiole and myristicin have been reported as inhibitors of aflatoxin G_1 production in *Aspergillus parasiticus* (Razzaghi-Abyaneh *et al*., 2007).

Both oxygenated phenylpropenes have an allyl side chain bonded to the aromatic ring which confers a lipophilic characteristic. Although not much is known about the mechanism of antifungal action of phenylpropenes, it has been reported that their lipophilic characteristic enables them to enter between the fatty-acid-chain constituents of the membrane lipid bilayers and alter the fluidity and permeability of the cell membranes (de Almeida *et al*., 2009). Remarkably, although the structural difference between *parsley*-apiole and myristicin is only seen in the presence of one methoxyl group on the aromatic ring, *parsley*-apiole was significantly more active than myristicin against *C. acutatum*. These results may suggest that electronic and/or steric factors in phenylpropenes might be important for antifungal activity. Further research to obtain information on the structure-activity relationship is required.

CONCLUSIONS

In the present study, the mycelial growth inhibition of *Colletotrichum acutatum* using essential oil obtained by hydrodistillation and four successive extracts (*n*-hexane, dichloromethane, ethyl acetate, and methanol) from fresh leaves and stems of parsley (*P. crispum* curly leaf) was studied. Essential oil and *n*-hexane extract exhibited the strongest antifungal activity against *C. acutatum*. GC/MS analysis demonstrated that the dominant components of both bioactive materials were phenylpropenes, specifically *parley*-apiole (or some isomer) and myristicin (or some isomer). Both constituents were isolated, and their structures unambiguously confirmed by spectroscopic methods. Then, antifungal activity against *C. acutatum* of these phenylpropenoids was evaluated. *Parsley*-apiole exhibited a strong fungistatic activity. Accordingly, *parsley*-apiole could be suggested as a structural template for developing new antifungal agents.

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Short article

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Common bean (*Phaseolus vulgaris* L.) yield response to chemical and biological fertilization in different localities of Colombia

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Respuesta productiva del cultivo del fríjol (*Phaseolus vulgaris* L.) a la fertilización química y biológica en zonas productoras de Colombia

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ABSTRACT

The research was carried out in 2015 in five bean producing locations in Colombia. The objective was to evaluate the effect of different treatments of chemical and biological fertilization on the yield of climbing and bush beans. According to the results obtained, in Antioquia, it is possible to replace the chemical fertilization by biological fertilization in the climbing bean Corpoica LAS-106 and the bush bean Citará. In Cundinamarca, similar situation was presented with "Cargamanto Rojo". In Santander, it was found that the combination of mycorrhizae (AMF) and *Rhizobium* improves the performance of the bush bean ICA-Rovirense (2839.5 t ha^{-1}) and ICA-Radical-J1J1 (1955.7 t ha^{-1}), with respect to national average yield (1.2 t ha^{-1}). The use of biofertilizers (mycorrhizal fungi and *Rhizobium*) improved the yields in the bean crop, with the materials used (climbing and bush) and in the localities studied. There is an additive effect in the applications of the biofertilizers evaluated, since it is a higher yield in the bean crop when these were applied individually combined.

Keywords:
Poultry manure
Mycorrhizae
Rhizobium
Phosphate rock

RESUMEN

La investigación se realizó en 2015 en cinco localidades productoras de frijol en Colombia. El objetivo fue evaluar el efecto de diferentes tratamientos de fertilización química y biológica sobre el rendimiento de frijoles volubles y arbustivos. De acuerdo con los resultados obtenidos, en Antioquia, es posible reemplazar la fertilización química por fertilización biológica en el frijol voluble Corpoica LAS-106 y el arbustivo Citará. En Cundinamarca, similar situación se presentó con el frijol "Cargamanto Rojo". En Santander, se encontró que la combinación de micorrizas (AMF) y *Rhizobium* mejora el rendimiento de los fríjoles arbustivos ICA-Rovirense (2839,5 t ha^{-1}) e ICA- Radical-J1J1 (1955,7 t ha^{-1}), con respecto al rendimiento promedio nacional (1,2 t ha^{-1}). El uso de biofertilizantes (hongos formadores de micorriza y *Rhizobium*) mejoró los rendimientos en el cultivo del frijol, con los materiales utilizados (volubles y arbustivos) y en las localidades estudiadas. Existe un efecto aditivo en las aplicaciones de los biofertilizantes evaluados, ya que se observó un mayor rendimiento en el cultivo de frijol cuando estos se aplicaron combinados que individualmente.

Palabras clave:
Gallinaza
Micorrizas
Rhizobium
Roca fosfórica

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Bean (*Phaseolus vulgaris* L.) is considered as one of the basic products of the peasant economy of small and medium-sized producers, located especially in the Andean region, with limitations in crop yields, high incidence of pests and diseases, and lack of good quality seeds (Pachón, 2010; Tofiño *et al*., 2016; FAO, 2016).

According to Fenalce (2016), in 2015 94,341 hectares of bean were planted in Colombia, with a yield of 101,497 tons; 93% of the plantations were located in the Andean region, 5.6% in the Inter-Andean Valleys, and 1.4% in the Caribbean region. Approximately, 65% of the national bean yield comes from the climbing varieties of cold and moderate cold climates, and the remaining 35% corresponds to bush varieties in medium and warm climates. In the other hand, 50% of production is concentrated in the departments of Antioquia, Santander, Huila, Tolima and Nariño.

In the tropics, the majority of the soils are from extremely up to strongly acid (pH<5.5), rich in exchangeable Al, and low in nutrients (Barber, 1995; Zapata, 2004; Toledo, 2012; Osorio, 2018), which restricts agricultural productivity. To compensate these problems, it is necessary to apply high amounts of lime and fertilizers, which increase yield costs (Osorio, 2018).

The medium-climate soils (1000-2000 m of altitude) in Colombia are of low to medium fertility and have some limitations for the normal growth of crops, since in general, their pH tend to be strongly acid (4.2-5.5), high in Al, low to medium of assimilable K (<0.3 $cmol_c\ kg^{-1}$), low in exchangeable Mg (<1.0 $cmol_c\ kg^{-1}$), and low in organic matter (<5%). The usable P content presents predominance towards low values (<15 mg kg^{-1}) (Tamayo and Osorio, 2014).

Moderate cold climate soils have high anionic exchange capacity, high phosphate binding, high organic matter content (<15%), but a very low mineralization of the organic matter (<1%). These soils have strongly acidic pH (4.0-5.5) and low levels of exchangeable bases, and in many cases, imbalances among the bases (Tamayo and Osorio, 2014).

It is considered that the use of biofertilizers could improve the quality of the soils and, therefore, the yield of the crops. Corpoica has been working with arbuscular mycorrhizal fungi (AMF), and bacteria of the genus *Rhizobium*, considered at the moment and worldwide, as biofertilizers for most of the crops. The combined use of both types of microorganisms, improves the yield conditions in more efficiently way than with the individual use of them, and are fundamental in the programs of integrated soil and crop management (Tamayo, 2016). According to what it was mentioned above, a study was established to determine the effect of the application of biofertilizers (mycorrhizal fungi and *Rhizobium*) on the yield of climbing and bush beans, in different producing localities of this specie in Colombia.

The use of microorganisms in the form of biofertilizers can have a positive effect on the yield in the bean crop in Colombia.

MATERIALS AND METHODS

The study was carried in 2015 (first semester), in different localities in the departments of Antioquia, Cundinamarca and Santander. In Antioquia, two experimental plots were established in two localities: CI La Selva (altitude of 2120 m, annual average temperature of 17 °C, average annual rainfall of 1850 mm, at 06°08'06''N, 75°25'03W), with climbing beans ICA-Viboral and Corpoica LAS-106 (both at a planting distance of 0.23 m among plants and 1.2 m among rows). The other locality was CI El Nus (altitude of 848 m, annual average temperature of 24 °C, average annual rainfall of 2500 mm, at 06°26'17.2''N, 74°49'32.1''W), with the bush bean varieties Quimbaya and Citará (both at a planting distance of 0.15 m among plants and 0.5 m among rows), in which it was evaluated the effect of several treatments with biological fertilizers. The treatments used were: Organic matter (poultry manure) (2 t ha^{-1}); *Rhizoglomus* sp. (15 g per plant), with a concentration of 250 spores g^{-1}, with 50 infective mycorrhiza propagules g^{-1}; commercial strains of *Rhizobium* (100 bacteria g^{-1}) (0.01 L per kg of seed) and/or with phosphate rock (PR) (600 kg ha^{-1}), in simple and combined form. All treatments received 20 g of KCl per linear meter, except for the chemical farmer treatment (control) (350 kg ha^{-1} of 10-30-10). Each experimental unit consisted of six rows, 10 m long and 0.6 m wide each. A randomized complete block experimental design with four replications, was used.

In Cundinamarca, a plot was established in the municipality of Pasca (at an altitude of 2180 m, annual average

temperature of 17 °C, an average annual rainfall of 1100 mm, at 4°18'27"N 74°18'03"W), with climbing bean "Cargamanto Rojo" (at a planting distance of 0.23 m among plants and 1.2 m among rows). The treatments used were: Organic matter (poultry manure) (2 t ha^{-1}); with *Rhizoglomus* sp. (15 g per plant), with a concentration of 250 spores g^{-1}, with 50 infective mycorrhiza propagules g^{-1}; commercial strains of *Rhizobium* (100 bacteria g^{-1}) (0.01 L per kg of seed) and/or with phosphate rock (PR) (600 kg ha^{-1}), in simple and combined form. All treatments received 20 g of KCl per linear meter, except for the chemical farmer treatment (control) (350 kg ha^{-1} of 10-30-10). Each experimental unit consisted of six rows, 10 m long and 0.6 m wide each. A randomized complete block experimental design with four replications, was used.

In Santander, two experimental plots were established in two localities: CI El Arsenal (altitude of 1450 m, annual average temperature of 22 °C, average annual rainfall of 1292 mm, at 6°40'05"N 72°42'00″W), with the bush bean variety ICA-Rovirense. The other locality was Curití (at an altitude of 1409 m, annual average temperature of 20 °C, an average annual rainfall of 1500 mm, at 6°36'16″N 73°04'05"W), with the bush bean variety ICA- Radical-J1J1. In both localitºies were used a planting distance of 0.15 m among plants and 0.5 m among rows, in which the effect of three strains of *Rhizobium* (P4, P8, and P14), and two inoculum of mycorrhiza MVA (*Rhizoglomus occultum* and *Acaulospora* spp.) were evaluated on the yield of bush bean varieties, ICA-Rovirense and Radical-JIJI. Each experimental unit consisted of six rows, 10 m long and 0.6 m wide. A randomized complete block experimental design with four replications was used. The study consisted in the evaluation of seven treatments; in Curiti was included a control, that involved the application of the combination of *Trichoderma lignorum*, and a mixture of mycorrhizal fungi (*Rhizoglomus occultum* and *Acaulospora* spp.), plus the inoculation of the seeds with *Rhizobium* sp.

RESULTS AND DISCUSSION

At C.I. La Selva, when performing a combined analysis during two consecutive cycles, in Corpoica 106 and ICA-Viboral, significant differences were present among treatments. As it can be seen in Table 1, the yield obtained with chemical fertilization in the variety Corpoica 106 was equal to that obtained with organic and biological fertilization, either with double inoculation, mycorrhizae plus *Rhizobium*, and/or with the application of PR. This agrees with several authors who report a positive effect of the different strains, both in their simple as in its combined forms, presenting the greatest effects when double inoculations were used (Saravesi *et al*., 2014; Galindo and Clavijo, 2009; Caldera *et al*., 2013; Clua *et al*., 2013; Granda *et al*., 2016; Romero *et al*., 2016).

Table 1. Yield of climbing beans Corpoica-LAS 106 and ICA-Viboral, during two consecutive cycles. CI La Selva.

Treatments	Corpoica LAS-106 (kg ha^{-1})	ICA Viboral (kg ha^{-1})
Control (chemical farmer treatment)	1527.0 a*	2033.2 a
Poultry manure plus inoculation with mycorrhizae	1602.9 a	1609.3 b
Poultry manure plus inoculation with *Rhizobium*	1439.6 a	1502.2 bc
Poultry manure plus phosphate rock	1585.1 a	1642.1 b
Poultry manure plus inoculation with mycorrhizae and *Rhizobium*	1480.3 a	1529.3 bc
Poultry manure plus inoculation with *Rhizobium* and phosphate rock	1518.2 a	1502.0 bc
Without poultry manure-inoculation with *Rhizobium* and mycorrhizae	1169.9 b	1157.0 c
CV	14.5%	22.0%

* Means followed by the same letter do not differ statistically at the level of 5%, according to Tukey's test.

It should be noted that the treatment with less yield was the one in which no poultry manure was applied, for both bean materials. This aspect is important due to the fact that, when the poultry manure is combined with inoculations of *Rhizobium* and mycorrhizae, the first one can act as a stimulator. In the variety Corpoica LAS-106, chemical fertilization can be replaced with the use of biofertilizers; contrary, the ICA-Viboral variety, responded better to the

application of chemical fertilizer (350 kg ha^{-1} of 10-30-10) than to the application of biofertilizers. According to Osorio (2016), arbuscular mycorrhizal fungi can improve the ability of the root system to absorb nutrients and improve the effect of phosphate amendments. Likewise, the rhizobium-legume association is considered a highly efficient process in biological fixation of atmospheric nitrogen (BNF); it is estimated that nitrogen fixation varies from 24 to 584 kg ha^{-1}; it has the capacity to supply up to 90% of the needs of the plant (Ángeles-Núñez and Cruz-Acosta, 2015). However, Osorio (2018) reports that there are differences in mycorrhizal dependence among cultivars of the same species.

The bean yield with this treatment was statistically superior to the different combinations of *Rhizobium*, phosphate rock and mycorrhizae (Table 1). As in the variety Corpoica LAS-106, in the ICA-Viboral, there was a significant response when poultry manure was applied, being this an activator of the biological-mineral fertilization. Organic fertilizers are a crucial element for the regulation of many processes related to agricultural productivity and can be a complement or replacement of synthetic fertilizers (Ramos and Terry, 2014). It may be argued that in the poultry manure, the microorganisms producers of organic acids can make more available the phosphate ions released from the phosphate rock, and thus improve yield (Osorio and Habte, 2013; Ramírez *et al*., 2013; Tamayo and Osorio, 2017). In both materials, yield per hectare during the two growing cycles was acceptable and, in some cases, exceeded the yields of the region (1.2 t ha^{-1}).

In medium climate conditions (CI El Nus) (Table 2), the Quimbaya and Citara bean materials showed a better yield response, when the control treatment (chemical) was applied, due to the soils of this area are low in fertility, very low in organic matter and exchangeable bases, and with acid pH. Although, it has been reported that in soils with very low organic matter there is a better response to organic applications than when applying chemical sources (Ramírez *et al*., 2008; Ramos and Terry, 2014; Hartmann *et al*., 2014; Guénon and Gros, 2015), In this study, the application of the biofertilizers was not enough to obtain the yields that were achieved with the chemical treatment, possibly because the applied doses were low. The treatments without poultry manure produced the lowest yields (936 and 352 kg ha^{-1}) for the bush varieties Citara and Quimbaya, respectively. A similar situation occurred in the locality of C.I. La Selva with climbing materials. The Quimbaya variety did not respond as significantly to the biological-mineral sources as did the climbing beans of moderate cold climate; however, there are reports of other mid-climate locations where their response has been positive. In all the varieties, the effect of the poultry manure on the yields is substantial (Table 2).

Table 2. Effect of biological-mineral fertilization on yield of bush bean in soils of medium climate. CI El Nus.

Treatments	Quimbaya (kg ha^{-1})	Citará (kg ha^{-1})
Control (chemical farmer treatment)	1264.0 a*	1583.9 a
Poultry manure plus inoculation with mycorrhizae	941.0 b	1400.0 ab
Poultry manure plus inoculation with *Rhizobium*	890.0 b	1022.8 bc
Poultry manure plus PR[2]	910.0 b	894.2 bc
Poultry manure plus inoculation with mycorrhizae and *Rhizobium*	850.0 b	755.6 c
Poultry manure plus inoculation with *Rhizobium* and PR	800.0 b	846.1 c
Without poultry manure–inoculation with *Rhizobium* and mycorrhizae	332.0 c	936.1 bc
CV	22%	12%

* Means with different letters in each column indicate significant differences according to the Tukey's test ($P \leq 0.05$).

In Pasca, the yield in the bean "Cargamanto Rojo" fluctuated between 568 and 2000 kg ha^{-1}. Table 3 shows significant differences among the treatments; the chemical farmer treatment (control), does not differ statistically from the treatments of poultry manure plus *Rhizobium*, phosphate rock and mycorrhizae and

Rhizobium. These yields, despite being from medium to low, the trend they present is the same of that obtained in C.I. La Selva with the ICA-Viboral bean, probably because poultry manure could improve the physical and biological conditions and help the activity of mycorrhiza and *Rhizobium* in the soil.

Table 3. Effect of organic and biological fertilization on the yield of climbing bean. Municipality of Pasca.

Treatments	Cargamanto Rojo (kg ha^{-1})
Control (chemical farmer treatment)	2000.0 a*
Poultry manure plus inoculation with mycorrhizae	941.0 bc
Poultry manure plus inoculation with *Rhizobium*	1199.0 abc
Poultry manure plus phosphate rock	1467.0 ab
Poultry manure plus inoculation with mycorrhizae and *Rhizobium*	1331.0 abc
Poultry manure plus inoculation with *Rhizobium* and phosphate rock	952.0 bc
Without poultry manure–inoculation with *Rhizobium* and mycorrhizae	568.0 c
CV	25%

*Means with different letters in each column indicate significant differences according to the Tukey's test ($P \leq 0.05$) (n=4).

In Table 4, it is observed that in the locality C.I. "El Arsenal", there were statistical differences ($P \leq 0.05$) among treatments, with a mean of 2354.5 kg ha^{-1} and a coefficient of variation of 14.67%.

The combination of AMF mycorrhizae (*Rhizoglomus* spp. + *Acaulospora*) and *Rhizobium* P14 strain, presented the highest yield (2839.5 kg ha^{-1}), while the lowest yield was shown in the treatment that included the *Acaulospora* combination and the *Rhizobium* P8 strain, with 1992.7 kg ha^{-1}. The combined use of both types of microorganisms (*Rhizobium* and mycorrhizal fungi) produces better effects than the individual use of them, the use of microorganisms in the form of biofertilizers improves soil nutrition and plant growth (Grageda *et al*., 2012; Elein *et al*., 2013; Lozano *et al*., 2015; Gardezi *et al*., 2016). In the locality of Curití, no significant differences among treatments were observed, in relation to the average yield of bean in kg ha^{-1}.

Table 4. Average yield (kg ha^{-1}) in Santander with regional bean varieties.

Treatments		CI El Arsenal ICA-Rovirense (kg ha^{-1})	Curití Radical-J1J1 (k ha^{-1})
Micorriza VA	*Rhizobium*		
Rhizoglomus spp.[1]	Strain P14	2086.4 cd *	1558.3 ab
Rhizoglomus spp.	Strain P4	2451.7 b	1918.0 a
Rhizoglomus spp.	Strain P8	2463.3 b	1827.3 ab
Acaulospora spp.	Strain P14	2338.4 bc	1488.0 ab
Acaulospora spp.	Strain P4	2309.4 bc	1782.3 ab
Acaulospora spp.	Strain P8	1992.7 d	1846.5 ab
Rhizoglomus spp + *Acaulospora*	Strain P14	2839.5 a	----
Acaulospora spp + *Trichoderma* + *Rhizoglomus* (control)		---	1955.7 a
CV		14.67%	14.09%

* Means with different letters in each column indicate significant differences according to the Tukey's test ($P \leq 0.05$) (n=4).
[1] *Rhizoglomus*=*Glomus* (Sieverding *et al*., 2014).

The highest effectiveness with respect to the yield was detected in the locality CI "El Arsenal", probably due to the quality of the organic medium, to the presence of existing beneficial microorganisms, which give the root greater capacity to explore the soil in search of water and nutrients, that results in higher yields; these results are similar to those obtained by Flores *et al*. (2014) in *Stylosanthes guianensis*. This allows to consider, that the combination of compost and biological agents in the management of the nutrition of the cultivated plants is beneficial, which represents for the farmers of bean, to produce more quantity and quality of food at a lower cost, preserving the environment.

CONCLUSIONS

The use of biofertilizers (mycorrhizae fungi and *Rhizobium*) improved yields in the bean crop, with the materials used (climbing and bush beans) and in the localities studied.

There is an additive effect in the applications of the biofertilizers evaluated, since a higher yield was observed in the bean crop when these were applied combined than individually.

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Determination of predominant soluble salts in soils of the irrigation district Alto Chicamocha of Boyacá

UNIVERSIDAD NACIONAL DE COLOMBIA

Determinación de sales solubles predominantes en suelos del distrito de riego Alto Chicamocha de Boyacá

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ABSTRACT

Keywords:
Anions
Cations
Geostatistics
E.C
Water table

In Boyacá, the Alto Chicamocha irrigation and drainage district (DRACH, by its initials in Spanish) is the department's main agricultural production unit, covering an area of 8016.78 ha and due to the natural conditions and the management that has been given to the high basin of the Chicamocha River, salinization has been recognized as a limiting factor**.** Therefore, we sought to determine the predominant soluble salts in the soils that comprise the DRACH. Based on the chemical soil analysis information of 301 samples, obtained from studies conducted by GISSAT-UPTC and Corpoica, E.C, pH, anions and cations present in the soil were determined. For the spatial analysis of the variables studied, the ArcGis 10.3 software was used. Thirty-one water samples were collected in wells of the phreatimetric network to carry out the chemical characterization of the water. It was found that 48.01% of the soils of the district were non-saline, 22.93% slightly saline, 14.74% moderately saline and saline 14.33%. The main soluble salts in the soil were Na_2SO_4, Ca_2SO_4, $NaCl_2$ and $CaCl_2$, which are related to the lacustrine origin and the presence of thermal springs in the region. The areas with greater problem of salinization occur in the municipalities of Tibasosa, Patrocinio, Ucaca, Las Vueltas; in Santa Rosa de Viterbo in the village of Salitre; in Duitama in the villages Cebadero and Higueras with E.C. greater than 2 dS m^{-1}.

RESUMEN

Palabras clave:
Aniones
Cationes
Geoestadística
C.E
Nivel freático

El distrito de riego y drenaje del Alto Chicamocha (DRACH por sus siglas en español), es la principal unidad de producción agropecuaria del departamento de Boyacá, Colombia, abarca un área de 8016,78 hectáreas y debido a las condiciones naturales y al manejo que se le ha venido dando a la cuenca alta del Río Chicamocha, la salinización ha sido reconocida como limitante. Por lo anterior se buscó determinar las sales solubles predominantes en los suelos que comprenden el DRACH. Con base en la información de análisis químicos de suelos de 301 muestras, obtenidas de estudios realizados por el GISSAT-UPTC y Corpoica, se determinó C.E, pH, aniones y cationes presentes en el suelo. Para el análisis espacial de las variables estudiadas se usó el sofware ArcGis 10.3. Se colectaron 31 muestras de agua en pozos de la red freatimétrica para realizar la caracterización química del agua. Se encontró que el 48,01% de los suelos del distrito eran no salinos, el 22,93 % ligeramente salino, el 14,74% moderadamente salino y salinos el 14,33%. Las principales sales solubles en el suelo fueron el Na_2SO_4, Ca_2SO_4, $NaCl_2$ y $CaCl_2$, las cuales están relacionadas con el origen lacustre y la presencia de termales en la región. Las zonas con mayor riesgo de salinización se presentan en los municipios de Tibasosa, veredas Patrocinio, Ucaca, las Vueltas; en Santa Rosa de Viterbo en la vereda Salitre; en Duitama en las veredas Cebadero e Higueras con C.E. mayores a 2,00 dS m^{-1}.

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Irrigation is considered a fundamental element in agriculture due to its effect on increasing production, improving the quality of products, sustainable intensification of land use and its contribution to safety food (FAO, 2000; 2011). In 2012, there were more than 324 million hectares in the world equipped for irrigation, of which approximately 85% or 275 million are effectively irrigated; by 2010, China became the country with the largest area of irrigation, and together with India, it covers 42% of the world's irrigation (AQUASTAT-FAO, 2014). In this way, irrigation has reduced the dependence on seasonal agriculture, thus achieving high agricultural production (Rhoades *et al*., 1992). In soils such as those in semi-arid regions where water requirements for crops are high and are necessarily supplied by irrigation districts, the use of these causes serious problems of environmental degradation that hampers the growth of crops and regional production in general (Abbas *et al*., 2013). Although irrigation districts bring advantages to agricultural systems, they have also brought with them environmental problems, such as: Salinization of soils, contamination of surface and groundwater bodies, changes in the landscape, and stress on plants.

The mobilization of salts in the soil is very variable among the different irrigation zones and according to Chedlia *et al.* (2012). Salinity depends on several factors such as the amount of salts present in the water, the texture of the soil, the distance from the intake to the district, the hydrogeology of the area, irrigation and drainage, and climatic conditions (rainfall regime, average temperature) (Duncan *et al*., 2008). For Girón-Ríos *et al.* (2009), salinity is a complex process of chemical degradation, which influences significant changes in the physical properties of soils and is affected mainly by the presence of salts in irrigation water and efficiency of the same (Aragüés *et al*., 2011).

Colombia has not been unrelated to the problem of salinization, in the different soil studies conducted, it is common to find that the soils have degradation phenomena, with erosion and salinization being the main problems in the territory. In this regard, the country ratified the United Nations Convention to Combat Desertification and Drought (UNCCD), which considers as an important strategy the identification and monitoring of soil degradation processes at the national, regional and local levels (FAO, 2000; 2011). Currently, public institutions of national order have been making important efforts to diagnose salinity problems in the country's soils, such as the national map of soil degradation by salinization carried out by IDEAM in 2017.According to this study, 14,041,883 ha (12.3%) of the country's soils (continental and insular areas) present some degree of degradation due to salinization. The very severe and severe degradation occupies 2,726,757 ha (2.4%); the moderate degree 8,885,369 ha (7.8%) and with 2,449,757 ha slightly (2.1%).

In Boyacá, the most important agricultural production unit in the department and the largest sprinkler irrigation district that has been built in a cold climate zone, is the DRACH, which has eleven irrigation units (Pacheco *et al*., 2004) and covers an area of 8016.78 ha. The problem of salinization has been recognized as a limiting factor in the agricultural soils that currently correspond to DRACH since 1960, the Colombian Institute for Agrarian Reform – INCORA (by its initial in Spanish), carried out drainage works to enable land to agriculture. The soils of the Tundama - Suamox valley are very vulnerable to salinization due to the natural conditions and the management that has been given to the upper basin of the Chicamocha River. For 2012 after an extreme rainfall weather event where 3000 ha were affected, the main victims were the agricultural producers who benefit from the DRACH and both urban and rural inhabitants of the municipalities of Paipa, Duitama, Nobsa, Sogamoso and to a greater extent Tibasosa (GISSAT, 2012), after this extreme weather event the problem of salinization was evidenced, where high electrical conductivities and saline scabs were observed. Therefore, with this research we sought to determine which were the most predominant soluble salts in the soils that comprise the DRACH and its special distribution.

MATERIALS AND METHODS

Location

The research was conducted in the DRACH which covers the towns of Paipa, Duitama, Tibasosa, Santa Rosa de Viterbo, Sogamoso and Nobsa (Figure 1) over the eleven irrigation units (Table 1).

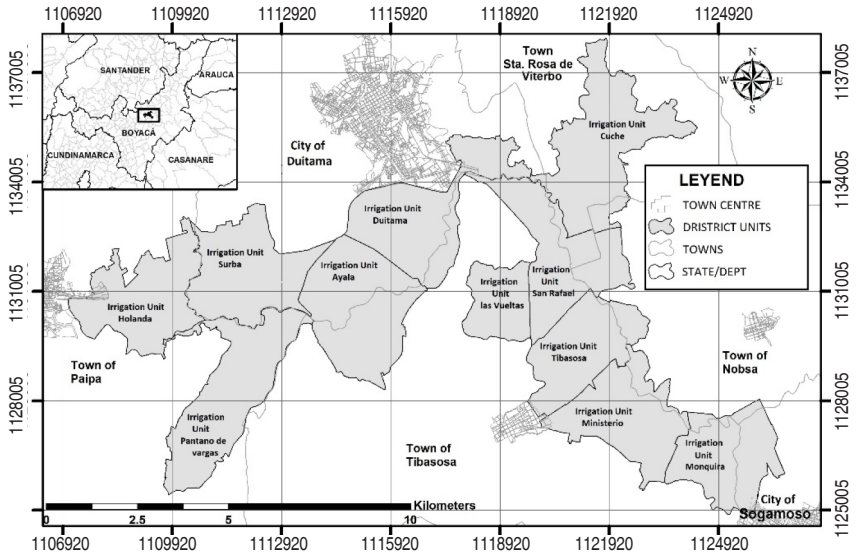

Figure 1. Location of the Irrigation and Drainage District of Alto Chicamocha.

Table 1. Irrigation units that define the DRACH.

N°	Irrigation Unit	Town	Rural áreas
1	Monquira	Tibasosa	Patrocinio
		Nobsa	Caleras
		Sogamoso	Siatame, Área Urbana
2	Ministerio	Tibasosa	Suescún, Centros, Patrocinio, Resguardo
		Nobsa	Ucuenga, Caleras
3	Tibasosa	Tibasosa	Suescún, Boyera, Centros, Área Urbana.
		Nobsa	Ucuenga
4	Las Vueltas	Tibasosa	Vueltas
5	San Rafael	Tibasosa	Peña Negra, Suescún
		Santa Rosa de Viterbo	Salitre
		Nobsa	Punta Larga, San Martín, Dicho, Ucuenga
6	Cuche	Santa Rosa de Viterbo	Cuche, Salitre, Cachavita, Creciente, Tunguaquita, La Chorrera
		Duitama	Tocogua
		Nobsa	San Martín
7	Duitama	Duitama	San Lorenzo de Abajo, Aguatendida, Tocogua, Área Urbana
		Tibasosa	Chorrito
8	Ayalas	Duitama	San Lorenzo de Abajo
		Tibasosa	Ayalas
9	Surba	Duitama	San Lorenzo de Arriba, San Lorenzo de Abajo
10	Holanda	Paipa	Toibita, Cruz de Bonza, Romita, Caños, Paipa Área Urbana
11	Pantano de Vargas	Paipa	Rincón De Vargas, Pantano De Vargas, Varguitas, Caños

The DRACHis located in the department of Boyacá, approximately 180 km from Bogotá, with an average temperature of 14 °C, with an average rainfall of 778 mm, an average altitude of 2560 m and a relative humidity of 70% (Martínez *et al*., 2008). Most of the soils are of agricultural vocation, however, dairy cattle stands out and, on a smaller scale, dual-purpose cattle (GISSAT, 2012).

In the DRACH data were taken for soils and waters which were georeferenced and projected to the Magna Colombia Bogotá coordinate system. The samples were faced in the dry period, after the La Niña phenomenon (2010-2012).

Soils analysis for Salinity

Based on the chemical soil analysis information from 301 samples, obtained from studies conducted by the Interinstitutional Research Group on Tropical Acid Sulphate Soils (GISSAT-UPTC, 2012) and Corpoica; electrical conductivity (E.C) by saturation extract – conductivity meter, hydrogen potencial (pH) by the 1:1 ratio method and the soluble salts in the soil were determined, for this the cations were obtained by atomic absorption.

To establish the spatial tendency of salinity, the geostatistical analysis of the E.C, pH, Sulfates ($SO_4^=$), Sodium (Na^{2+}), was carried out. For the spatial analysis of the variables studied, the Geostatistical analyst tool of the ArcGis 10.3 software was used, the interpolation method used was ordinary Kriging. To classify the salinity of DRACH soils, the following ranges of electrical conductivity (E.C.) were used: saline ($\geq$ 2 dS m^{-1}), moderately saline ($\geq$ 1.5 < 2 dS m^{-1}), slightly saline ($\geq$ 1 < 1.5 dS m^{-1}) and not saline (< 1 dS m^{-1}), according to Castro and Gómez (2010). Regarding the classification used for sulfates ($SO_4^=$), the following ranges were used: Under 11 ppm, Optimum 38 ppm and High 64 ppm according to Castro and Gómez (2010). For sodium, the ranges were used: low 0.1 cmol kg^{-1}, medium 0.1-0.5 cmol kg^{-1} and high > 0.5 cmol kg^{-1} according to Castro and Gómez (2010).

Groundwater analysis for Salinity

Thirty-one water samples were collected wells of the phreatimetric network to perform the chemical characterization corresponding to pH with potentiometer, E.C with conductivimeter, sulfates by colorimetry, and by titulation were obtained the chlorids, carbonates, Bicarbonates and nitrates, the cationes (Na^{2+}, Ca^{2+}, Mg^{2+} and K) by atomic absorption. This information allowed to determine the quality of these waters and their possible relationship with the salinization processes. The samples were analyzed in the soil and water laboratory of the Faculty of agricultural sciences of the UPTC (by its initials in Spanish), with the analytical methodology (Analytical Control Soil Laboratory- ICONTEC).

The research had a non-experimental design, since independent variables could not be manipulated, where the phenomenon of salinization was observed in its natural context within the irrigation District. For each of the variables, the basic descriptive statistics were calculated, in which the mean, median, kurtosis and asymmetry were calculated, as well as the parameters of the semivariogram (Sill, Nugget and Range) and the cross validation (Table 2).

RESULTS AND DISCUSSION

As it is mentioned by Narváez et al. (2014), classical statistics and geostatistics are some of the tools for the analysis of soil salinity that contributes to the identification of affected areas and the monitoring of spatio-temporal variations, in the Table 2 the results for the geostatistical analysis of the variables studied are observed.

The statistical model that was best adjusted for the variables pH (Figure 2), E.C (Figure 3), Na^{2+} (Figure 4) was the exponential, while for the sulfates was the Rational Quadratic model (Figure 5). These variables were studied because they are the most representative soluble salts of the zone.

According to the Kolmogorov-Smirnov normality test, the variables analyzed show a non-normal behavior except for pH (Table 2). For Cressie (1993), normality is not a mandatory requirement for the analysis of geostatistical data, however, it must be considered that the distribution of the data don´t show a very long tail, since it compromises the results for the Kriging estimates.

Variability is an intrinsic characteristic of each property and its specific behavior for each soil condition, use and

Table 2. Descriptive and spatial statistics of the variables studied.

Statistics \ Variables	E.C.	pH	Na^{2+}	$SO_4^=$
Mean	1.5	5.411	4.446	2.456
Sill (CO+C)	0.64	0.9483	1.119	0.909
Range	2640.84	1280	3055.31	3874.58
Nugget (CO)	0.35	0.0531	0.725	0.494
Cross-validation (Error mean)	0.0178	-0.03	0.010	-0.079

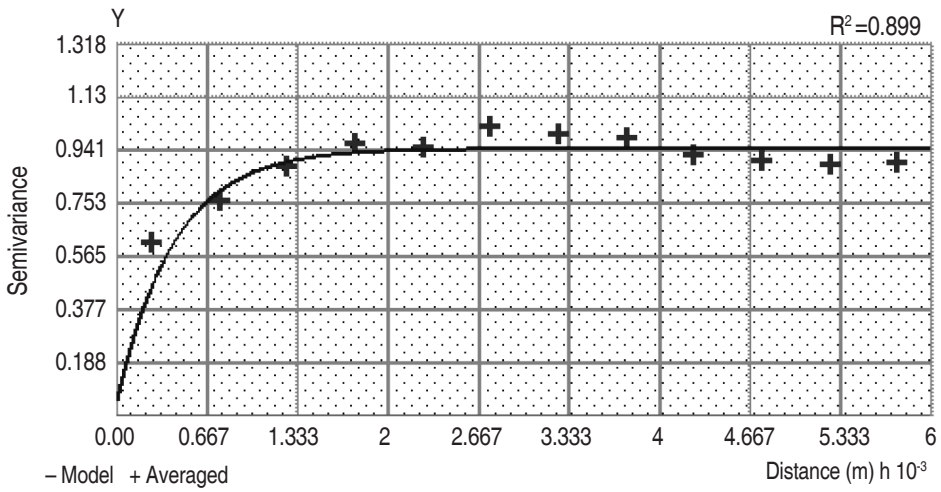

Figure 2. Semivariogram of pH.

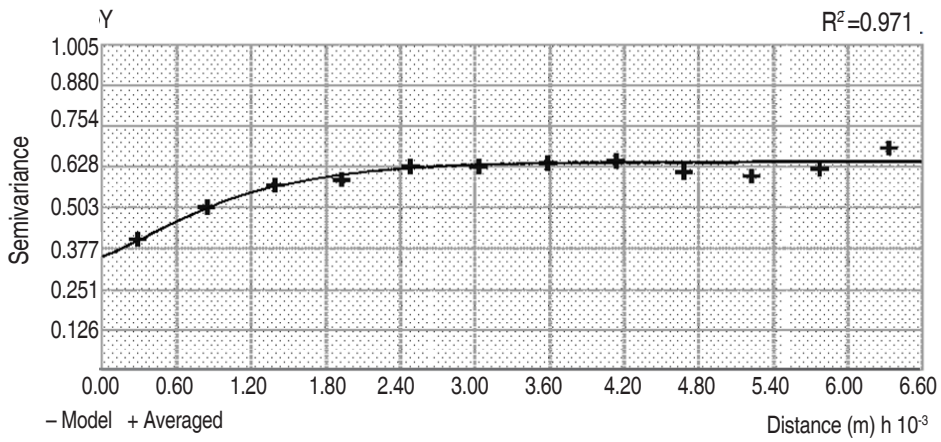

Figure 3. Semivariogram of E.C in the DRACH.

management; therefore, interpreting the autocorrelation of a variable along its distribution in one area, and its correlation with respect to another variable or variables, is a study that has a large number of immersion factors, and requires clear knowledge of the factors that condition it (Jaramillo *et al.,* 2008). The Range is the zone of influence and corresponds to the distance from which two observations are independent (Giraldo, 2002).

All the variables had a moderate spatial dependence (Table 2) according to the classification proposed by Cambardella *et al.* (1994), since the relationship between the plateau and the nugget effect [C/(Co+C)] is between 0.25 and 0.75, the pH showed a range of 1287.93 m, the E.C a range of 2048.9 m, Na^{2+} a range of 3055.31 and the $SO_4^=$ a range of 3874.58, indicating that there is a spatial correlation in each of the variables at that distance.

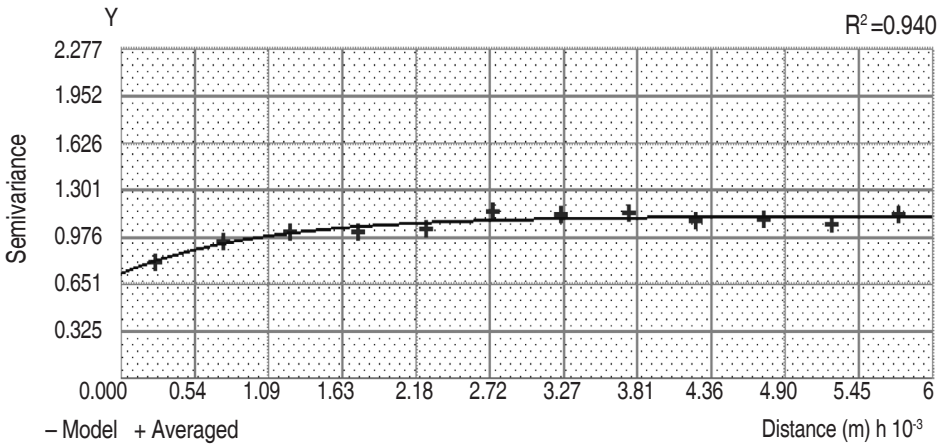

Figure 4. Semivariogram of Na^{2+}

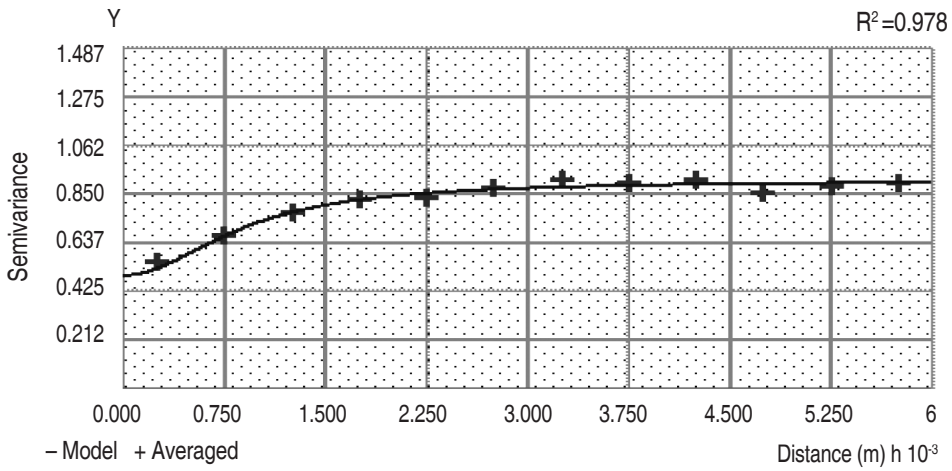

Figure 5. Semivariogram of $SO_4^=$

The results of the descriptive analysis for the soil variables showed that the E.C. of the soil saturation extract, presented an average value of 1.77 dS m-1; the highest value was registered in the town of Santa Rosa rural area "El Salitre" with an E.C. of 6.57 dS m-1 and the lowest in the municipality of Paipa with 0.16 dS m-1. Figure 6 shows the behavior of the mean of the E.C. values for the districts that make up the district.

It should be noted that 48.01% of the district's soils are found in the range of E.C. < 1 dS m^{-1}, which classifies it as non-saline (Table 3), 22.93% between ≥ 1.0 < 1.5 dS m^{-1} which is classified as slightly saline, 14.74% between ≥ 1.5 < 2 dS m^{-1} which are moderately saline and 14.33% have values higher than ≥ 2 dS m^{-1} are saline soils (Figure 7), which indicates that they are soils that begin to have salt problems, according to Castro and Gómez (2010). These values coincide with those reported by Mercado *et al.* (2011) who in the Doctrina Irrigation District, Colombia found values higher than 3 dS m^{-1}, which indicated salinity problems for those areas. The electrical conductivity of the soil is a function of the clay content

and water content (Kurtulus *et al*., 2009, Narváez *et al*., 2014), with the topography, and the phreatic level which allows high variability to be present for this parameter (Ulset *et al*., 1998).

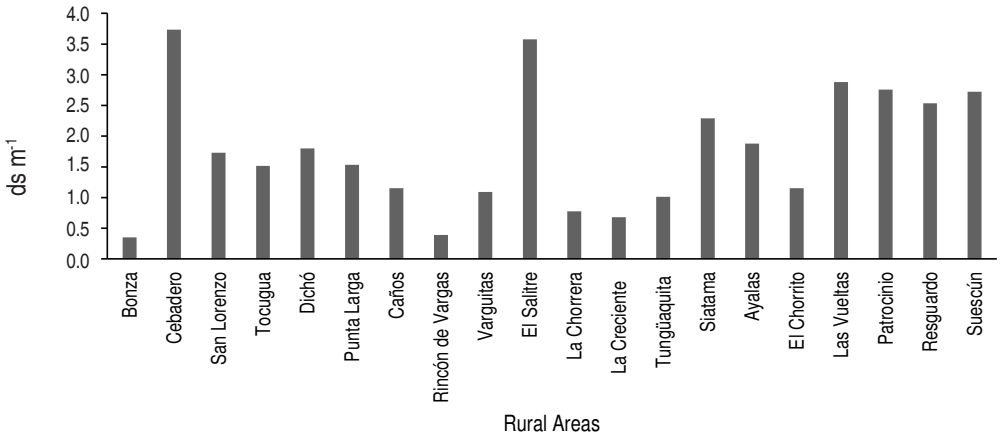

Figure 6. General average of the E.C. in the soils of rural DRACH.

Table 3. E.C. Ranges for DRACH soils.

E.C. Range (dS m^{-1})	Area (ha)	Percentage (%)
< 1.0	3848.84	48.01
≥ 1.0 < 1.5	1838.09	22.93
≥ 1.5 < 2.0	1181.34	14.74
≥ 2.00	1148.51	14.33
Total	8016.78	100

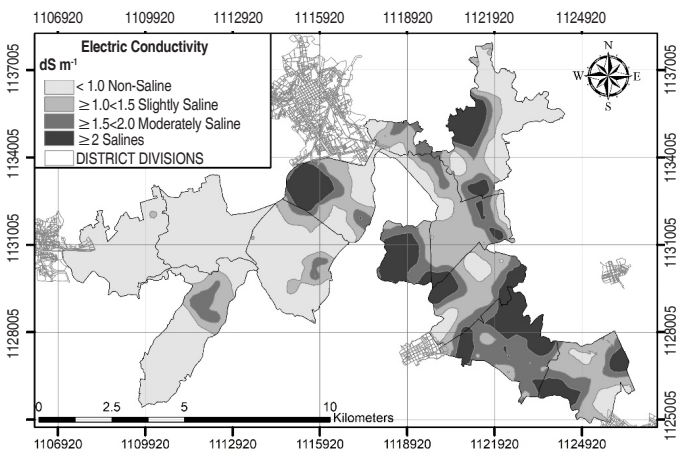

Figure 7. Spatial distribution of E.C in the DRACH soils.

The pH presented an average value of 5.52. In general, it was between the range of 5-6 that corresponds to moderately acid soils, with 46.64% of the total area of the district (Figure 8). The *Resguardo* area had a mean value of 7.28, that may be associated with the presence of salts such as sodium chloride ($NaCl_2$) (Mercado *et al.*, 2011).

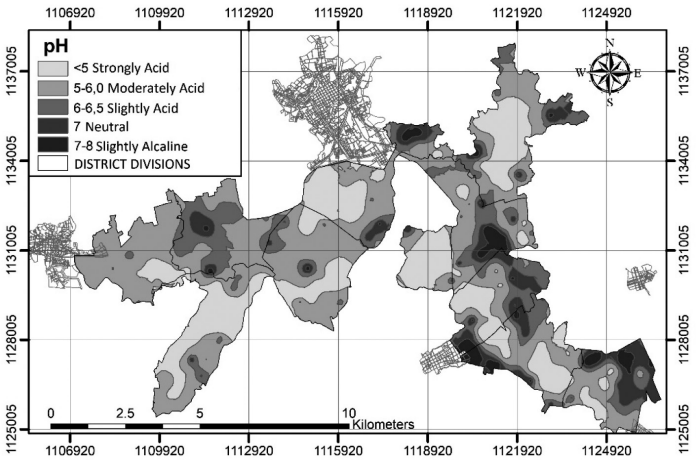

Figure 8. Spatial distribution of pH in the DRACH soils.

For the cations, it was evidenced that the element with the greatest presence in DRACH soils was sodium, followed by Ca in less quantity. Considering the ranges provided by Castro and Gómez (2010), for Na greater than 0.5 cmol kg^{-1} and for Ca higher than 6 cmol kg^{-1}, there is a high grade of salinization due to very high concentration levels. In Figure 9, the behavior of Na within the district is observed, 73.93% of the soils have low concentrations with levels of 0.1 cmol kg^{-1}, 21.49% are in the middle range with values between 0.1-0.5 cmol kg^{-1} and 4.57% have high levels with values greater than 0.5 cmol kg^{-1}. High values were found in the Salitre area with 4.29 cmol kg^{-1}, followed by El Cebadero with 4.085 cmol kg^{-1} that may be related to the saline deposits (Na_2SO_4 and $NaCl_2$), which are of the characteristics of the thermal sources of Paipa (Moreno and Fechi, 2006). Taiz *et al.* (2006), mention that the Na concentration of a sodium soil can not only directly damage the plants, but also degrade the structure of the soil, reducing the porosity and being a highly hygroscopic element, it traps the water molecules of the soil, which causes the water of hydration to decrease for other nutrients, also affecting the structure of the soil by breaking up its particles (Madueño *et al.*, 2006). Besides that the hydrolysis of the sodium clays, leads to the alkalinization of the profile, and these cause an intense mineral alteration (Mata *et al.*, 2014). While if Ca^{2+} and soluble salts are very abundant in the soil, it is likely that the profile is very poorly differentiated, but its structure tends to be stable, as a result of the flocculating action of Ca^{2+} so the high osmotic pressure of the soil solution is responsible for low productivity (Mata *et al.,* 2014).

Ca^{2+} reported high values in the Salitre rural zone with 42.01 cmol kg^{-1}, followed by las Vueltas with 30 cmol kg^{-1} while the lowest values were found in the village of Rincon de Vargas with 4.236 cmol kg^{-1}. Mercado *et al.* (2011) obtained Ca values higher than 6 cmol kg^{-1} in an average of 89.5% of the total area studied.

The sulphates ($SO_4^=$) were the dominant anions, mainly due to their high solubility, the area of Cebadero presented an average of 93.298 mg kg^{-1} being the highest value, followed by the area of Salitre with 54.238 mg kg^{-1}; they are high values considering the ranges established by Castro and Gómez (2010). In Figure 10, the behavior of the sulphates in the district is observed, 48.90% of the area is at a low level (11 ppm), 48.86% of the area presents an optimum range with 38 ppm and the 0.30% of the district presents problems due to high levels (64 ppm).

For chlorides (Cl^-) the rural area *el Resguardo* reported the value of the highest average with 42.80 mg kg^{-1}

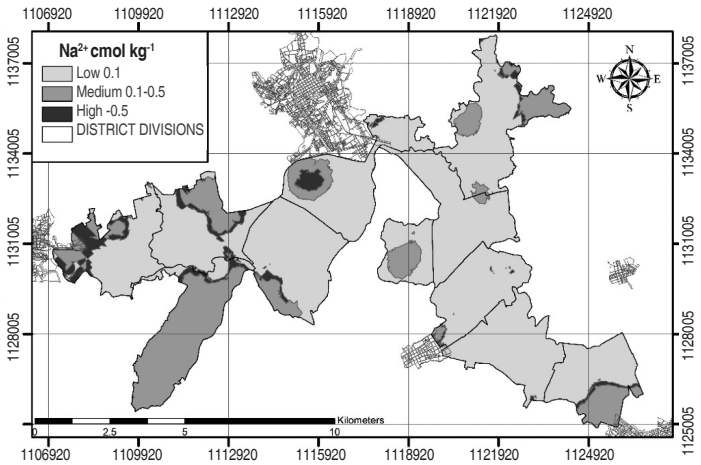

Figure 9. Spatial distribution of Na^{2+} in the DRACH soils.

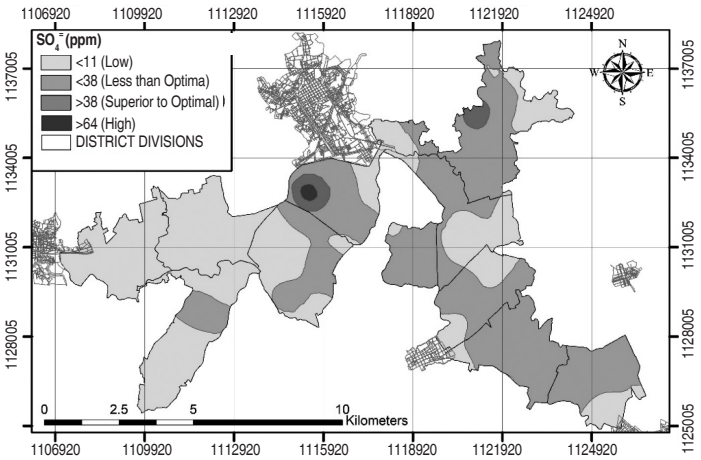

Figure 10. Spatial distribution of $SO_4^=$ in the DRACH soils.

followed by Siatame in Sogamoso with 30.76 mg kg^{-1} the behavior of these is noted in Table 3. These latter are at a low level according to Castro and Gómez (2010), where the high ranges are 142 mg kg^{-1} and above. Qadir *et al.* (2007) state that the predominant soluble salts in saline soils are sulfates, chlorides, bicarbonates of Na^{2+}, Ca^{+2} and Mg^{+2}. Na is the cation that most binds to sulfates and chlorides to form salts, with less frequency found potassium and bicarbonates, carbonates and nitrates (Dorronsoro, 2011; Ramírez, 2011).

Fernández *et al.* (2007) indicate that the high variability of the parameters mentioned above may be due to the fact that soil properties such as interchangeable cations, sulphates and others not related to soil morphology are affected by the use and / or management. Similarly, Narváez *et al.* (2014) indicate that in saline soils, the depth of the water table, the evapotranspiration rate, and other geohydrological factors impact on the chemical properties, resulting also in a high spatial and temporal variability of the same. According to this, the high variability obtained for the parameters is generated by the interaction of the different properties of the soil with its immediate environment, this means, agricultural practices, variations in climate among others, agricultural practices, variations in climate among others, cause

changes at different scales (Mallarino and Wittry, 2004). For the waters, it was found that the water tables oscillated between 0.54 and 2.99 m in the irrigation units San Rafael in Nobsa and Cuche in Santa Rosa de Viterbo; these values coincide with those reported by Castro and Gómez (2015) who argue that in the dry season about 30% of the area, the reading and analysis levels of the District's aeration profile zone are between 0.0 and 1.0 m, while in the critical rainy season there is a rise in the water table, reaching times of flooding, which can affect up to 45% of the area. It is worth noting that the waters are frequented by sales of soluble, the upper basins of the Chicamocha River have geological units composed of a very important lithological record, mostly composed of sedimentary rocks with some outcrops of igneous rocks in the Paipa and Iza surroundings (Moreno and Fechi, 2006). The phreatic mantles are found on calcareous materials can cause salinization, if these are located in climates, they are characterized by values of evapotranspiration superior to those of precipitation, the phreatic levels can ascend by the capillarity to the surface of the soils (IGAC, 2012).

Water quality analysis for irrigation was carried out in 31 wells (Figure 11). According to the classification of waters by to the Riverside standards (US Salinity Laboratory - USLS), all of them have problems of salinity and sodium (Olías *et al*., 2005). The average value of the E.C was 3.12 dS m^{-1}, for pH was 6.34. In this regard, Taiz *et al*. (2006) mention that the higher the concentration of salts in water, the greater the electric conductivity and the lower the osmotic potential (the higher the osmotic pressure). The above allows to relate the E.C. of the soil with the water used for irrigation, in some units such as Ministry where E.C of 4.29 dS m^{-1} was observed in the soil and 9.79 dS m^{-1} in the water; in Cuche with 4.66 dS m^{-1} in water and 5.64 dS m^{-1} in soil, and finally in the irrigation unit of Vueltas with 8.54 dS m^{-1} in water and 2.6 dS m^{-1} on soil, indicating that water used for irrigation may be contributing to the salinization of soils in the district.

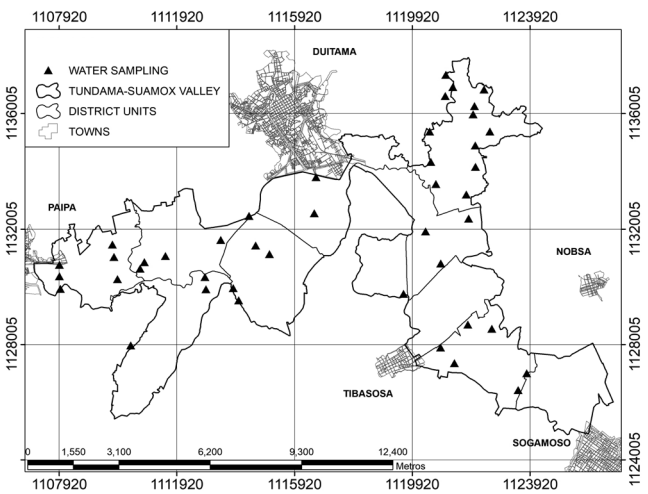

Figure 11. Spatial location of the water sampling points in the DRACH.

According to investigations by Alfaro and Ingeominas (2010), in Paipa springs there are problems with sulphated waters, as well as high concentrations of Na^{2+}, K, Ca^{+2}, Mg^{+2}, Cl^{-1}, $SO_4^{=}$, HCO_3. For Porras (2010), in the present thermal manifestations, alkaline-chlorinated waters appear, probably these are related to deep hydraulic circuits (reservoir), sulphate-alkaline waters that owe their origin to the addition of alkaline sulphates to the preceding waters, the area is characterized by the predominance of essentially Cretaceous and Tertiary sedimentary rocks, and the presence of possible volcanic necks with an approximate age of 2.5 million years, in addition there are thermal manifestations of high temperature, probably due to the presence of an

acidic magmatic intrusion, located at a depth of 5 km. There is possible a presence of two thermal aquifers in granular and silicic sedimentary rocks with predominant secondary permeability is highlighted, in addition, the Honda or Río Salitre stream carries a high quantity of salts, supplied by the thermal springs that appear in the middle and lower area of the sub-basin (POT Paipa, 2012). Moreno and Fechi (2006), affirm that the presence of thermomineral waters in the Paipa sector are affecting the DRACH soil, due to the fact that at present they are drained to a great extent to the Vargas canal, generating: disaggregation of soil particles caused by sodium sulphates, ionic contamination by chlorine and sodium, mainly due to inadequate handling of thermal or sulfuric waters (IGAC, 2012).

CONCLUSIONS

The main soluble salt present in the soil was sodium sulphate (Na_2SO_4), followed by calcium sulfate (Ca_2SO_4), sodium chloride ($NaCl_2$) and calcium chloride ($CaCl_2$), which are highly soluble and toxic for the plants.

It was found that the areas with the highest problem of salinization are in Tibasosa in Patrocinio, Ucaca, Las Vueltas zones; in Santa Rosa de Viterbo in the Salitre zone and finally in Duitama in Cebadero and Higueras zones, as they have electrical conductivities greater than 2 dS m^{-1}.

The groundwater of the sector plays an important role in the dynamics of salinization of the DRACH, due to the connection with the upper basin of the Chicamocha River, which presents geological units composed of a very important lithological record.

The presence of the Paipa springs with sulfated water problems and high concentrations of cations and anions strongly influence the salinization of DRACH soils.

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Comparison of soil use in the infiltration of rainwater: pasture and forest

UNIVERSIDAD NACIONAL DE COLOMBIA

Comparación del uso del suelo en la infiltración del agua de lluvia: pastos y bosque

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Keywords:
Forest fragmentation
Flooding
Climate change

ABSTRACT

The lowlands of the forests of Itajaí/SC, Brazil were extensively and predominantly fragmented into urban settlements, port facilities, and rice plantations. In addition to climate change events and existing environmental conditions, the region is susceptible to flooding. Consequently, this study aims to analyse the hydrologic functioning of the lowland forests in the infiltration of rainwater in Itajaí. A map of land use and occupation was created using the Geographic Information System – GIS, and crossed with the soil maps of the city. Two areas with the same soil classifications were selected; a pasture area and a forest area. In August and October, these areas were sampled and classified according to the following criteria: grain size, moisture, permeability, and organic matter content of the soils. The infiltration rate of the soils in the sample units was tested using the double ring infiltrometer. Analysis of variance (ANOVA) was used to verify the correlation between the obtained values. The average values for the samples obtained in areas without vegetation were 3.45 cm h^{-1} and 3.60 cm h^{-1} in August and October, respectively. In the area with forest vegetation, the average values were 19.05 cm h^{-1} and 8.70 cm h^{-1} for the samples obtained in August and October, respectively. Although the soil conditions were the same, this study found significant differences in the water infiltration rates in the soil surface between the forest areas and the areas without vegetation. The forest vegetation denotes its potential role in the infiltration of rainwater in the floodplain of area.

Palabras clave:
Fragmentación del bosque
Inundaciones
Cambio climático

RESUMEN

Los bosques de Itajaí/SC, Brazil han sido intensamente fragmentados en la llanura, reflejando en el predominio de asentamientos urbanos, estructuras portuarias y cultivos de arroz. Al sumarse los eventos provenientes del cambio climático a las condiciones ambientales existentes, la región se torna susceptible a las inundaciones. De esta forma, este trabajo tiene como objetivo analizar la función hídrica de los bosques de llanura en la infiltración del agua de las lluvias en Itajaí. Se utilizó el Sistema de Información Geográfica para la elaboración de un mapa de uso y ocupación del suelo y se comparó con el mapa de suelos del municipio. De estos análisis fueron seleccionadas dos áreas en la llanura en la misma clase de suelos, una con pastoreo y una cubierta por bosque. En los meses de agosto y octubre, estas áreas se muestrearon y clasificaron acorde a los siguientes criterios: granulometría, humedad, conductividad hidráulica y contenido de materia orgánica de los suelos. También en estas unidades de muestra se realizó el ensayo de velocidad de infiltración básica (VIB) mediante el método de anillos concéntricos. Se utilizó el análisis de varianza (ANOVA) para verificar el efecto entre las variables obtenidas. Los valores medios obtenidos de VIB para área de pastoreo fueron 3,45 cm h^{-1} y 3,60 cm h^{-1} para los meses de agosto y octubre, respectivamente. En el área con vegetación se obtuvieron valores medios de 19,05 cm h^{-1} y 8,70 cm h^{-1} para los meses de agosto y octubre, respectivamente. En este trabajo fue posible verificar que hay diferencias significativas en la infiltración de agua, en la parte superficial del suelo, entre las coberturas con bosque y con pastoreo, donde las condiciones edáficas son iguales. La vegetación forestal denota su papel potencial en la infiltración de agua de lluvia en la planicie de inundación del área.

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The consequences of climate change in Latin America and the Caribbean, according to Herrán (2012), are reduced availability of water, diminished performance of agriculture in low altitude areas, loss of biodiversity, and increase of flooding and droughts.

Between 1992 and 2001, floods were the most frequent natural disaster (43% of 2257 disasters) and affected more than 1.2 billion people all over the world in a decade. These floods are the result of climate change associated with the impact on ecosystems (Millennium Ecosystem Assessment, 2005). In recent years, climate change has been aggravated by natural or anthropogenic causes, or a combination of these (Moraes *et al*., 2015).

According to Frank (1995) the region of Vale do Itajaí, in Santa Catarina, and the municipality of Itajaí in particular, have a history of floods and flooding due to environmental characteristics. The most devastating floods in the region occurred in 1983, 1984, 2001, 2008 and 2011 (Santos *et al*., 2012). The last flood in the city occurred in 2015. Combined with the fact that Itajaí is located on a plain that comprises the mouth of river Itajaí and that it receives the energy and matter inputs of the entire river basin, the forest fragmentation and urbanization of this region increases its susceptibility to floods in rainy periods (Marenzi, 2012). According to Tucci (2008), floods occur in two ways: the riverbanks, because of the temporal and spatial variability of rainfall and runoff in the river basin; and due to urbanization, which occur in urban drainage because of the effect of waterproofing the soil or preventing the flow of water.

Itajaí is located in the Atlantic Forest and it is represented by the Montane Atlantic Forest and Lowland Atlantic Forest (Veloso *et al*., 1991). The lowland coastal forest was extensively fragmented and mostly occupied by settlements, port structures, and agricultural and pasture systems, especially rice crops in the rural area (Marenzi, 2012).

Several works have been carried out to better understand the impacts of urban planning on the hydrological cycle (Barron *et al*., 2013; Braud *et al*., 2013; Davies *et al*., 2008; Miller *et al*., 2014; Randhir and Raposa, 2014; Rougé and Cai, 2014; Sillanpää and Koivusalo, 2015; Suriya and Mudgal, 2012; Yang *et al*., 2014; Dalagnol *et al.,* 2017; Santos *et al.,* 2017) and the effects of irrigated agriculture (Foster *et al*., 2015; Blainski, *et al.,* 2016). Some works specifically sought to analyse the influence of vegetation on water infiltration (Alves *et al*., 2005; Costa *et al*., 1999; Godim *et al*., 2010; Nunes *et al*., 2012; Sidiras and Roth, 1987; Touma and Albergel, 1992; Zwirtes *et al*., 2011; Rossato *et al.,* 2016). However, no works on the subject in the region of Vale do Itajaí, in Santa Catarina were identified.

Based on the history of physical and material damages caused by flood phenomena in Santa Catarina, specifically in the Itajaí Valley, there is a need for research on measures to minimize this trend (Moraes *et al*., 2015). Therefore, this work sought to verify the influence of vegetation cover on the rate of infiltration and physical characteristics of soil inherent to infiltration. Consequently, the aim of this study is to analyse the physical and hydrological characteristics of soil in pasture and forest areas and understand the functional role of forests to provide information on floods in Itajaí.

MATERIALS AND METHODS

The study area is located in the municipality of Itajaí, in the geographical coordinates 26°54'28''S and 48°39'42''W, at an altitude of 2.0 m. Research was conducted from May 2014 to February 2015.

According to the Köppen classification, the regional climate is humid with a warm summer (Cfa). Annual average rainfall in the region is 1545.3 mm, average temperature is 20.3 °C, and average relative humidity is 83.73%. The heaviest rainfall occurs from January to March and from October to December and the lowest rainfall occurs from April to June and July to September (Araújo *et al*., 2006).

Google Earth images were used to classify soil use and occupation and select the sample areas and ArcMap 10.0 software was used to georeference a scene from the urban part of the municipality, with information from the database of the Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI, 2004). For the other scenes, we created a mosaic using Regeemy software 0.2.43 (Fedorov *et al*., 2003). ArcMap 10.0 software was used for supervised classification.

The experimental areas were selected according to similar environmental characteristics, like soil, climate and relief

although one area consisted of secundary atlantic forest (21.44 ha) and the other consisted of artificial pastures (15.63 ha) with semi-extensive livestock farming. Both areas are situated along the right bank of river Itajaí - Mirim and are classified as Histosols (PMI, 2006).

Two sampling campaigns were executed, one in August (dry season) and one in October (rainy season) of 2014. Soil was collected from four random sampling points in each area to determine moisture content, organic matter content, and grain size. The basic infiltration rate was determined at the location.

Each sample contained five kilos of soil at a depth of 50 cm. For the hydraulic conductivity analysis, soil was collected at a depth of 50 cm to obtain an undisturbed sample of 15 cm in height and 10 cm in diameter.

The basic infiltration rate test was run according to the methodology double ring, described by Bernardo *et al.* (2013), using I=K*T^A, where K and A are constant and depend of the soil. Moisture content was analysed according to EMBRAPA (1997). This methodology was used for grain size analysis of soils rich in organic matter. Fifty grams of soil were weighed in a beaker and portions of oxygen peroxide (H_2O_2) were successively added to the soil. The samples were heated to accelerate the reaction and stirred using a glass rod to observe the effervescent reaction. The addition of H_2O_2 was suspended and the samples were covered with a clock glass and left to sit overnight. This process was repeated another two times. To remove the excess of H_2O_2, water was added to the beaker instead of washing the sample filter paper, as indicated in the methodology. The samples were left to sit overnight, after which the supernatant was withdrawn and the water was restored. Also, this procedure was repeated two more times. The material was put to dry in an oven at 105 ºC for 24 h. The material was buffered and 20 g were weighed and sieved (#0.063 mm) with the addition of water. The retained material was collected and dried in an oven at 105 ºC for 24 h. The weight difference was used to calculate the percentage of silt/clay and, consequently, of sand. Organic matter content of the soil samples was determined using the methodology described by Wright (2008). Permeability was determined using the variable load methodology described by Das (2006).

For the analysis of variance, the following factors were considered: 1) soil cover (forest and pasture); 2) campaign, both considered orthogonal and fixed. The analysed variables were: basic infiltration rate, hydraulic conductivity, moisture content and organic matter content. Normality was verified using the Kolmogorov-Smirnov test and the homogeneity of variances was verified using the Cochran test; when necessary, transformations to Log10 (X+1) were applied (Underwood, 1997).

RESULTS AND DISCUSSION

The municipality of Itajaí presented fairly fragmented areas, mainly by agricultural activities and urban pressure (Figure 1). We defined the following six classes of soil use and occupation: culture, urban patch, reforestation, exposed soil, forest, and water. Once the study area was selected, the following classes were specifically verified: cultivation, exposed soil and vegetation, with a predominance of the former, mainly consisting of pasture for cattle farming. However, this type of farming is not prevalent in the municipality; irrigated agriculture represents most of the cultures in the municipality, occupying 9283 ha, with a production of 69,000 t of grain (EPAGRI, 2010).

The analysis of soil use and occupation showed that the municipality of Itajaí has small portions of forest in the lowlands. According to Bedin (2013), since the 1970s, the urban fabric of Itajaí has expanded rapidly and lots have replaced cultivated areas. Ribas (2013) stresses that in 1985, the cultivation and reforestation areas occupied most of the municipality. Around 1995, the forest cover increased in sparse fragments, possibly resulting from the abandonment of part of the agricultural areas. The urban area also expanded due to the economic dynamics of the region. This expansion caused the process of forest fragmentation, especially in plains that are most sought after for urban expansion, and intensified the problem of soil sealing and flooded areas in periods of intense rainfall. These facts support the importance of understanding the functional role of forest cover in these flood plains. This considering the natural vulnerability to flooding due Itajai location at the mouth of a large river basin (Santos *et al*., 2012; Moraes *et al*., 2015). Natural vulnerability intensifies with the urbanization process (Tucci, 2008). It is also important to consider that climate change intensifies vulnerability, because in many parts of the world there is a significant increase in temperature, resulting in

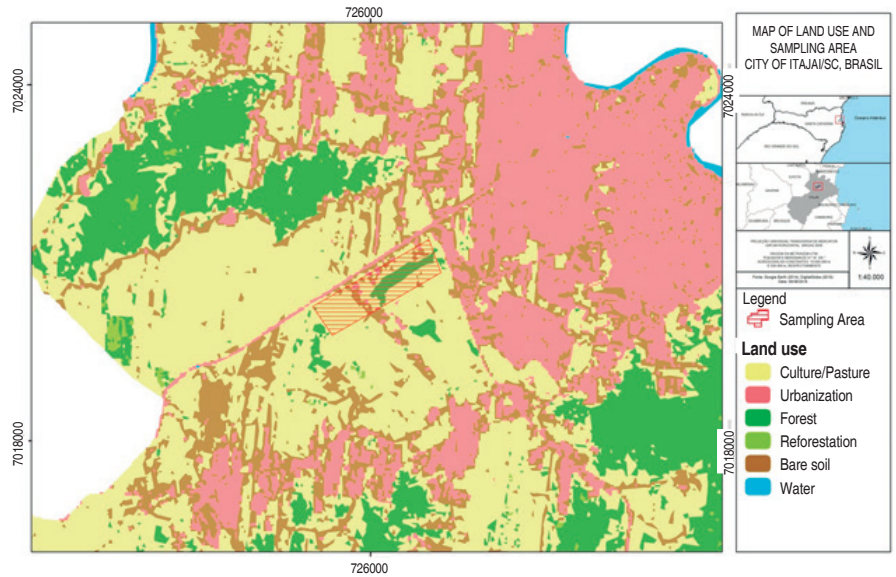

Figure 1. Use and occupation of the municipality of Itajaí (SC), detail of the study area.

extreme events. (Righi and Robaina, 2010; IPCC, 2014), such as floods. The scientific community has reinforced the conviction that climate variability has intensified in recent decades as a result of human activities (Moraes *et al*., 2015). All the analysed samples contained more than 99% of clay. Organic matter content ranged from 13.4 to 51.7%, moisture content ranged from 59.8% and 84.5%, the basic infiltration rate ranged from 0.6 and 39 mm h^{-1}, and conductivity ranged from 0.05 and 1.13 cm h^{-1} (Table 1).

Table 1. Observed values for the variables hydraulic conductivity, moisture content, organic matter content, basic infiltration rate and grain size for two soil covers in Itajaí (SC).

		Hydraulic conductivity		Moisture content		Organic matter content		Basic infiltration rate		Grain Sixe	
		Campaign									
	Sample No.	1	2	1	2	1	2	1	2	1	
		(cm h^{-1})		(%)				(mm h^{-1})		% Clay	% Sand
Cover											
Pasture	1	0.21	0.77	80.0	64.8	38.5	46.4	1.2	3.6	99.9	0.1
	2	0.85	0.02	76.0	64.0	37.4	40.1	7.2	3.6	99.8	0.2
	3	0.28	0.32	77.2	59.8	25.9	38.6	4.8	4.8	99.3	0.7
	4	0.59	0.66	84.5	66.9	49.3	31.7	0.6	2.4	99.7	0.3
	Average	0.48	0.44	79.4	63.9	37.8	39.2	3.4	3.6	99.7	0.3
	Min.	0.21	0.02	75.9	59.8	25.9	31.7	0.6	2.4	99.3	0.1
	Max.	0.85	0.77	84.5	66.9	49.3	46.4	7.2	4.8	99.9	0.7
Forest	5	0.18	0.75	68.6	68.4	37.5	34.3	18.0	3.6	99.8	0.2
	6	0.05	1.13	82.6	59.9	27.0	46.0	39.0	24.0	99.8	0.2
	7	0.13	0.19	81.3	61.9	13.4	46.6	4.8	1.2	99.9	0.1
	8	0.30	0.17	79.3	62.9	34.1	51.7	14.4	6.0	99.8	0.2
	Average	0.16	0.56	77.9	63.3	28.0	44.6	19.0	8.7	99.8	0.2
	Min.	0.05	0.17	68.6	59.9	13.4	34.3	4.8	1.2	99.8	0.1
	Max.	0.30	1.13	82.6	68.3	37.5	51.7	39.0	24.0	99.9	0.2

The differences found for the factor soil cover with the variable basic infiltration rate corroborate the findings of Alves *et al.* (2005) and Zwirtes *et al.* (2011), considering the most of basic infiltration rate in the soils of the forest. However, the values found in the forest area by these authors are significantly higher (116.5 cm h^{-1} and 122.48 cm h^{-1}, respectively). This difference can be associated with the type of studied soil. Histosol or organic soil have hydromorphic organic horizons and these horizons remain saturated with water during a period of the year (Zanella *et al.*, 2018). Therefore, with lower infiltration rate if compared another soils. The moisture content of the soil in the two areas was slightly different, with smaller amounts in the forest area. This can be explained by the fact that larger vegetation areas require more water (Raven, 2007).

The analysis of variance indicated significant differences in relation to cover for the infiltration rate (Table 2). The significantly higher values were in forest areas regardless of the time of the year (Figure 2). The significantly higher moisture content was in campaign 1 for both types of cover (Figure 3).

Table 2. Significance value and F value of the variables: hydraulic conductivity, moisture content, organic matter content and basic infiltration rate obtained in the August and October campaigns of 2014 for different soil covers (forest and pasture), in the city of Itajaí (SC).

	Basic infiltration rate		Moisture content		Organic matter		Hydraulic conductivity	
	P	F	P	F	P	F	P	F
Campaign	0.281	1.2748	<0.0001	47.458	0.057	4.418	0.305	1.145
Cover	0.041	5.250	0.641	0.229	0.625	0.251	0.553	0.372
Campaign Coverage*	0.268	1.351	0.848	0.038	0.102	3.132	0.204	1.807

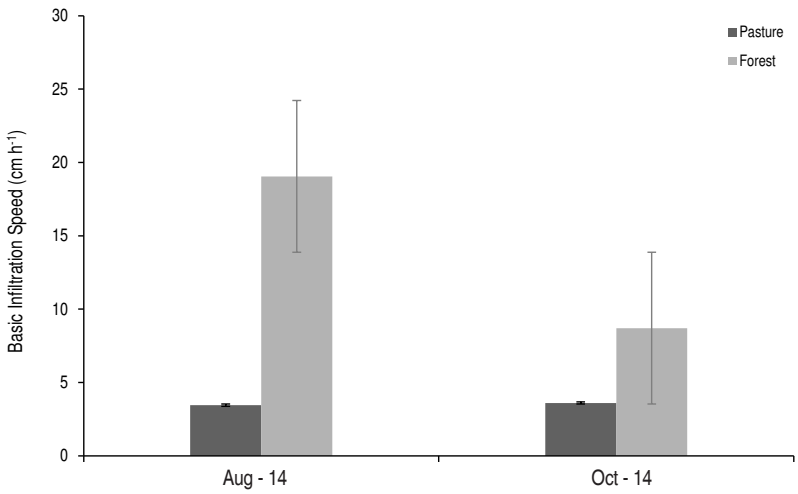

Figure 2. Basic infiltration rate, average and respective standard error, obtained in the August and October campaigns of 2014 for different soil covers (forest and pasture), in the city of Itajaí (SC).

The analysis of variance proved to be significant for the factor campaign and can be explained by the occurrence of increased rainfall in the period. However, there were no significant differences between the soil covers, which suggests the same moisture condition of the analysed soil samples. According to Araújo and Reis (2014), the accumulated volume in August (118 mm) was higher than the accumulated volume of October (63 mm). Therefore, the result of greater moisture in the first campaign is expected.

The high values of clay in all the analysed samples suggest that any difference between the studied areas can be the result of the forest function on the soil, especially in terms of aeration due to the root system (Gliessman, 2000).

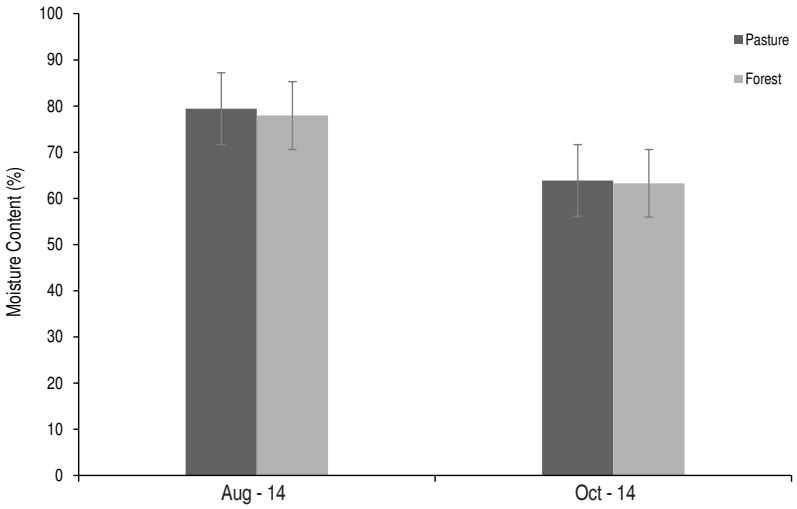

Figure 3. Moisture Content, average and respective standard error, obtained in the August and October campaigns of 2014 for different soil covers (forest and pasture), in the city of Itajaí (SC).

The content of organic matter was numerically smaller in the forest area of the first campaign. This fact cannot be explained since the forest generates the most amount of organic matter, as verified by Cerri *et al.* (1991) and Leite *et al.* (2003), even considering recycling. However, the increase in organic matter content may derive from the semi-extensive livestock farming in the area and the faeces residue. In the October campaign, the organic matter content was numerically greater in the forest area, which is closer to the expected result.

The hydraulic conductivity results followed the same trend of the organic matter content, according to which the presence of cattle could have been more frequent in August (first campaign) since the semi-extensive system generates organic waste that may have contributed to the hydraulic conductivity. In the October campaign, the forest cover was compensated in organic matter and therefore provided greater hydraulic conductivity. Significant correlations were observed between the organic matter and the physical attributes of the soil (Beutler *et al.*, 2001) since organic matter contributes to the increase in the porosity of soil (Khaleel *et al.*, 1981; Logan and Harrison, 1995), and therefore facilitates permeability.

These results indicate the possibility of greater contribution of forest compared to pasture to minimize flooding in Itajaí. Thus, conservation actions and educational programs or actions are extremely important, because they can minimize environmental problems, especially those associated with the silting of the river and soil sealing (Moraes *et al.*, 2015).

CONCLUSIONS

The water infiltration was significantly higher in the presence of forest vegetation (P=0.041) indicating its potential role in the infiltration by rainwater absorption in the floodplain of Itajaí/SC, Brazil. In this way, the pressure for occupation urban and agriculture stresses the importance of protecting the last forest remnants.

It is important to consider that sampling in one area limited the study, since other samples could confirm the value of the forest in the infiltration of the water in the soil, minimizing the problems of floods, common in the region and intensified by events due to climate change. In any case, the result of this study indicates the importance of transforming the last forest remnants into Protected Areas.

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Effects of land-use change on Nitisols properties in a tropical climate

UNIVERSIDAD NACIONAL DE COLOMBIA

Efectos del cambio de uso de la tierra en las propiedades de los Nitisoles en un clima tropical

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ABSTRACT

Land use change, especially conversion of native forests to cultivated land, exerts an impact on the physical, chemical and hydrophysical soils properties. To quantify and better understand responses, this study was aimed at evaluating the influence of different tropical soil management systems reflected in some physic, chemical and hydro-physical properties. Nine Nitisol profiles were evaluated and grouped in three categories: (I) native forest (Benchmark > 30 years); (II) soils formerly cultivated then turned to pasture (Conservation > 10 years); and (III) soils under continuous cultivation (Agrogenic > 50 years). The analyzed variables were organic matter, bulk density, soil particle density, porosity, field capacity, texture and structural index. Results determine that the action of traditional farming techniques in tropical environments produces excessive soil degradation. Organic matter content and the structural index showed a linear relationship with high degree of dependence (R^2=0.99). Bulk density average for (I) and (II) profile were lower (P<0.05) than the bulk density values for (III). In the regression analyses the bulk density increased, the field capacity decreased, and the tendency for profile (I) and (II) were of a linear type. While the profile for (III) was of a polynomial type with (R^2=0.83), being able to be influenced by the higher values of bulk density, greater soil compaction, lower structural index, organic matter and porosity in correspondence with the other profiles.

Keywords:
Degradation
Physical properties
Soil management
Tillage

RESUMEN

El cambio en el uso de la tierra, especialmente la conversión de bosques nativos en tierras cultivadas ejerce un impacto sobre las propiedades físicas, químicas e hidrofísicas de los suelos. El objetivo de este estudio fue evaluar la influencia de diferentes sistemas de manejo de un suelo tropical reflejado en algunas de sus propiedades físicas, químicas e hidrofísicas. Se evaluaron nueve perfiles de un suelo Nitisol agrupado en tres categorías: (I) bosque nativo (Referencia > 30 años); (II) suelos anteriormente cultivados y luego convertidos en pastizales (Conservados > 10 años); y (III) suelos bajo cultivo continuado (Agrogénicos > 50 años). Las variables analizadas fueron materia orgánica, densidad aparente, densidad real, porosidad, capacidad de campo, textura e índice estructural. Los resultados determinaron que la acción de las técnicas agrícolas tradicionales en ambientes tropicales produce una excesiva degradación de las propiedades del suelo. El contenido de materia orgánica e índice estructural muestran una relación lineal con un alto grado de dependencia (R^2=0,99). La densidad aparente promedio para los perfiles (I) y (II) fue menor (P<0,05) que los valores de densidad aparente del perfil (III). En los análisis de regresión a medida que aumenta la densidad aparente disminuye la capacidad de campo y la tendencia observada para (I) y (II) es lineal, mientras que para (III) es polinómica con (R^2=0,83), pudiendo estar influenciado por los valores más altos de la densidad aparente, mayor compactación, menor índice estructural, materia orgánica y porosidad en correspondencia con los otros perfiles.

Palabras clave:
Degradación
Propiedades físicas
Manejo del suelo
Labranza

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Since the beginning of agricultural development, during Neolithic era, humans influenced the properties of virgin lands. This land use is one of the main drivers of many processes of environmental change, as it had influenced basic resources within the landscape. Without a doubt, the anthropogenic factor has played its role as a force that affects both soils and climate, having increased exponentially during the last 300 years, to such an extent that this phenomenon is now considered a new era in the evolution of the Earth: "The Anthropocene" (Crutzen and Steffen, 2003). During the last millennium, nearly all soils have undergone some modifications through human action, directly or indirectly. Thus, at present, the genesis of many soils has been determined more from human effects than from natural forming factors. Such is the magnitude of this transformation that some authors speak of an agrogenic evolution of soils (Lebedeva *et al*., 2008).

Tropical regions are characterized by having high rainfall intensities resulting in severe erosional rates if inappropriate land management practices are applied. In Cuba, there are extensively weathered tropical soils (Ferralsols, Nitisols, Oxisols, Lixisols, Acrisols, and Alisols)". It is a country with soils subjected to anthropogenesis, constituting one example for tropical regions. I Cuba being a long-narrow island presents diverse soil groupings, dominated by the Nitisols, especially in the western and center of the country, which because of its good characteristics, has been the main source of food production for the inhabitants of this region. (Hernández *et al.*, 2017). In the last 10 years some Cuban researchers has been working on the diagnosis for soil degradation, in order to achieve their sustainability through medium and long-term research on the basis of the so-called reference sectors (Hernández *et al*., 2006). Some of these results were presented by Hernández *et al*. (2009, 2013) and by Olivera (2017), about the change of Red Lixiviated Ferralitic soils properties (Nitisols) by continued cultivation. These authors show indicators and mechanism of soil degradation as well as agroproductive responses to improvement.

Changes in soil functionality are indicators of their performance and handling to which they are subjected. However, it should be noted that if a better understanding is to be achieved then studies of each soil type must be conducted., It is also noted that among soil samples with similarities in their relationships and some dependency, which makes it possible for the interpretation of one, to infer the behavior of another (Fultz *et al*., 2013; Duval *et al*., 2016). According to information from the Soil Institute of Cuba (2006), 69.6% of soils have low organic matter (OM) and 43.3% have strong erosion, limiting their productivity. In this sense the pursuit of quality of soil and timely identifying their limitations is one of the main objectives of research in soil physics, as demonstrated by (Zhu *et al*., 2015; Deng *et al*., 2016). Although significant knowledge about soil and landscape change in agriculture has been gained, much remains unknown and uncertain. Major reasons are the relative scarcity of soil studies, methodological shortcomings, the complexity of agricultural systems and soils, and imprints of multiple land use and environmental change. With this purpose, the aim of this study was to evaluate the influence of different tropical soil management systems reflected in their physical, chemical and hydro-physical properties.

MATERIALS AND METHODS

The study was carried out in Banao community in the Sancti Spiritus Province, which is located to the south in the central region of Cuba, between X: 216 000 - 218 000 and Y: 539 000 - 550 000 according to the rectangular plane coordinate system of Northern Cuba, cartographic sheet 4 281-II-b (Figure 1). This experimental area ("Hermanos Alonsos") has suffered anthropic action for more than 30 years, despite the high income generated by the production of onions (*Allium cepa* L.). The production levels obtained in the last years have compromised the ecosystem's stability and as a vulnerable factor increased soil erosion (Figure 2).

We analyzed nine Nitisol profiles where three conditions were considered as treatments (three repetitions for each land use) grouped as: (I) native forest (Benchmark > 30 years); (II) soils formerly cultivated then turned to pasture (Conservation > 10 years); and (III) soils under continuous cultivation (Agrogenic > 50 years). The profile description was according to the manual for detailed cartography and integral evaluation of soils (Hernández *et al*., 1995). Soils were identified by the World Reference Base for Soil Resources (WRB) classification (International Union of Soil Sciences (IUSS), Working Group, 2014).

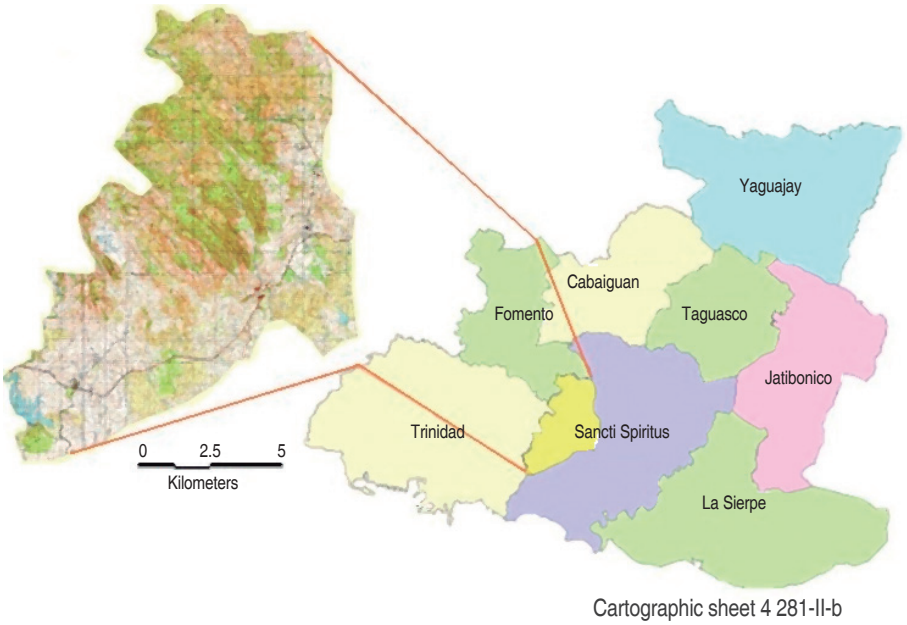

Figure 1. Cartographic sheet generated from the Map Info Professional 9.0 software (2010) (Banao comunity).

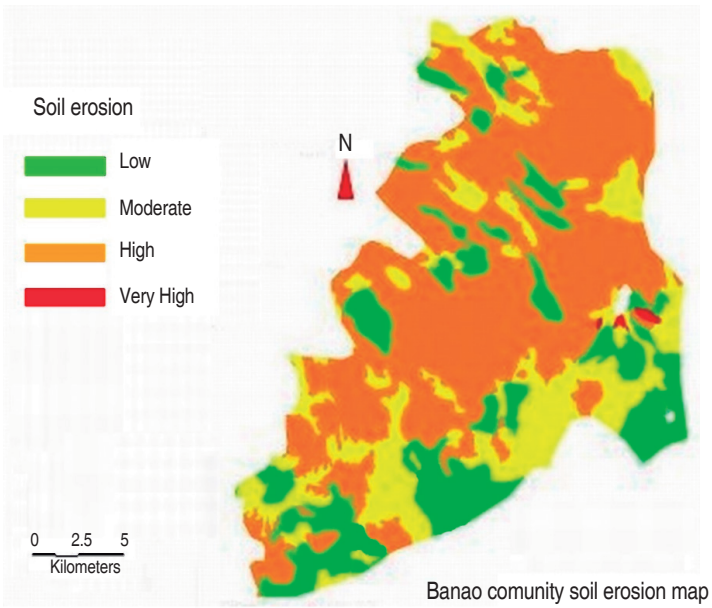

Figure 2. Soil erosion using the 1:25 000 map generated from the MapInfo Professional 9.0 software (2010).

Soil samples were taken every 10 cm up to 50 cm deep for every profile. All soil samples were analyzed at the Territorial Station of Investigations of the Sugar Cane (ETICA), Villa Clara, Cuba, in accordance with the following analytical methods: organic matter (OM) according to (Walkley and Black, 1934); color profile (Figure 3) by (Munsell Soil Color Charts, 2000), bulk density (Db) was determined for a humidity close to the field capacity considering that some soils contain dilatable clays in which the humidity level exerts a big influence on their values. (Db) was measured using 100 cm^3 cylinders (Grossman and Reinsch, 2002) and soil

particle density (Dp) was determined according to the pycnometer method. Field capacity was obtained from periodical measurements of soil water content (Simeon, 1979). Soil texture Hydrometer Method (Bouyoucos, 1951) and structural index was determined according to Pieri (1995). The total porosity (P) was calculated using the mathematical model represented below [1].

$$\%P = \left(1 - \frac{Db}{Dp}\right)100 \qquad [1]$$

Benchmark profile | Conservation profile | Agrogenic profile

Figure 3. Nitisol profile under different management systems.

Variance analysis (ANOVA) tests were carried out on the results considering a completely random design in which the profiles of each category were considered repetitions and each of the three conditions were considered treatments. Physical soil measurements were compared by the Tukey test, at 5% probability. Relationships between bulk density, field capacity, organic matter and structural index were investigated by regression models. All statistical analyses were performed using the statistical software Agroestat (Barbosa and Maldonado, 2011).

RESULTS AND DISCUSSION

The results demonstrated that the soils in this study presented differences in their physical properties due to its land utilization (Table 1). According to (Hernández *et al*., 2010), soil degradation processes increase where the management practices are selected without regard for the edaphological conditions, which are specific to every particular region. Benchmark topsoil (0 – 10 cm) had a mean (± standard deviation) bulk density of (0.90 ± 0.09 g cm^{-3}), which was similar to that of topsoils taken from conservation profile (0.94 ± 0.06 g cm^{-3}). The mean bulk density for Benchmark and Conservation profile were lower (P<0.05) than the bulk density values for agrogenic profile (1.21 ± 0.04 g cm^{-3}). Bulk density values both for Benchmark and Conservation profile are considered normal, whereas for the agrogenic profile the soils are considered compacted according to Martin and Duran (2011). A tendency towards a lower bulk density for soils taken from Benchmark and the conservation profile system compared to those from agricultural land uses (agrogenic) could also be detected for the 10-50 cm (Table 1). Compaction, measured by increased bulk density, often accompanies structural degradation as soil porosity and pore size are reduced through physical compression and decreased organic matter. According to Homburg and Sandor (2011) in the Mimbres study, bulk density increased about 9% relative to uncultivated soils and may not have been a major problem, but that degree of compaction has been shown in experiments to inhibit maize seedling development and root elongation, especially in fine-grained soils.

Soil particle density (Pd) follows a similar behavior in all profiles without revealed significant differences. This could be explained because the soil macro particles can

Table 1. Land use systems effect on soil physical properties.

Depth (cm)	Db (g cm^{-3})	Dp (g cm^{-3})	Porosity (%)
Profile I (Benchmark)			
0-10	0.90 ± 0.09^{b}	2.61 ± 0.2025^{a}	65.5 ± 1.8036^{a}
10-20	0.98 ± 0.11^{b}	2.64 ± 0.2042^{a}	62.9 ± 3.1262^{a}
20-30	1.03 ± 0.07^{b}	2.72 ± 0.1778^{a}	62.1 ± 4.1356^{a}
30-40	1.04 ± 0.08^{b}	2.76 ± 0.2402^{a}	62.3 ± 2.8219^{a}
40-50	1.05 ± 0.10^{b}	2.77 ± 0.1670^{a}	62.1 ± 2.5696^{a}
Profile II (Conservation)			
0-10	0.94 ± 0.06^{b}	2.63 ±0.22^{a}	64.3 ± 5.14^{a}
10-20	1.02 ± 0.05^{b}	2.68 ± 0.15^{a}	61.9 ± 1.81^{a}
20-30	1.05 ± 0.07^{b}	2.76 ± 0.27^{a}	61,9 ± 2.81^{a}
30-40	1.05 ± 0.07^{b}	2.72 ± 0.18^{a}	61.4 ± 4.35^{a}
40-50	1.10 ± 0.06^{b}	2.74 ± 0.16^{a}	59.9 ± 2.70^{a}
Profile III (Agrogenic)			
0-10	1.21 ± 0.04^{a}	2.80 ± 0.24^{a}	56.8 ± 1.00^{b}
10-20	1.26 ± 0.05^{a}	2.79 ± 0.15^{a}	54.8 ± 1.64^{b}
20-30	1.31 ± 0.07^{a}	2.79 ± 0.19^{a}	53.1 ± 1.95^{b}
30-40	1.39 ± 0.12^{a}	2.76 ± 0.15^{a}	49.6 ± 5.77^{b}
40-50	1.38 ± 0.07^{a}	2.78 ± 0.19^{a}	50.4 ± 2.25^{b}

Average ± standard deviation. Different letter in the same column vary for (P<0.05).

change in a short time with the management practices, but it does not happen in the same way with microparticles and soil texture. On the other hand, soil porosity did not present significant differences between the benchmark and conservation profile, but with the agrogenic profile. Higher bulk densities are usually attributed to compaction, structural damage and destruction of macropores of topsoil by overgrazing and use of machinery (Kelishadi *et al*., 2014; Lal, 1986; Price *et al*., 2010) or by intensive agricultural practices (Emadi *et al*., 2008). Our results are thus consistent with previous studies that reported higher bulk densities for pastures or croplands compared to native or forest plantations in Argentina, the north-eastern USA (Zhou *et al*., 2008), Costa Rica (Reiners *et al*., 1994), Iraq (Emadi *et al*., 2008) and Ethiopia (Selassie and Ayanna, 2013).

According to the regression analyses, as the bulk density increases, the field capacity decreases and the observed tendency is linear for Benchmark and Conservation soils (R^2=0.94) and (R^2=0.93) respectively (Figure 4A, B). That is, the field capacity decreases 42.6% for Benchmark profile and 49.1 % for Conservate profile for each unit of soil bulk density variation. It a polynomial regression can be observed For the Agrogenic profile (R^2=0.83) (Figure 4C). This non-linear behavior corresponding to the Agrogenic profile could be influenced by higher values of bulk density, presenting a greater soil compaction, lower structural index, organic matter content and porosity coinciding with a compacted soil according Martin and Duran (2011) as was previously described.

The texture of all land types and depths were predominantly clayey, with the clay content increasing with depth (Table 2). The Soils of all depths sampled were classified as (Nitisol Rodico Eutrico) based on (IUSS, 2014). On average, soils from the lower depths (25–50 cm) contained slightly less sand and slightly higher clay content (Table 2). According to Homburg and Sandor (2011) structure degradation is signaled by loss of granular structure, weaker structure

grade, tendency toward larger aggregates (peds) and massive condition. These differences appear to be due to intensive anthropogenesis experienced by the soils which was not sub soiled in the last years. It is therefore reasonable to infer that the soil compaction is conditioned by the history of land use and management. According to

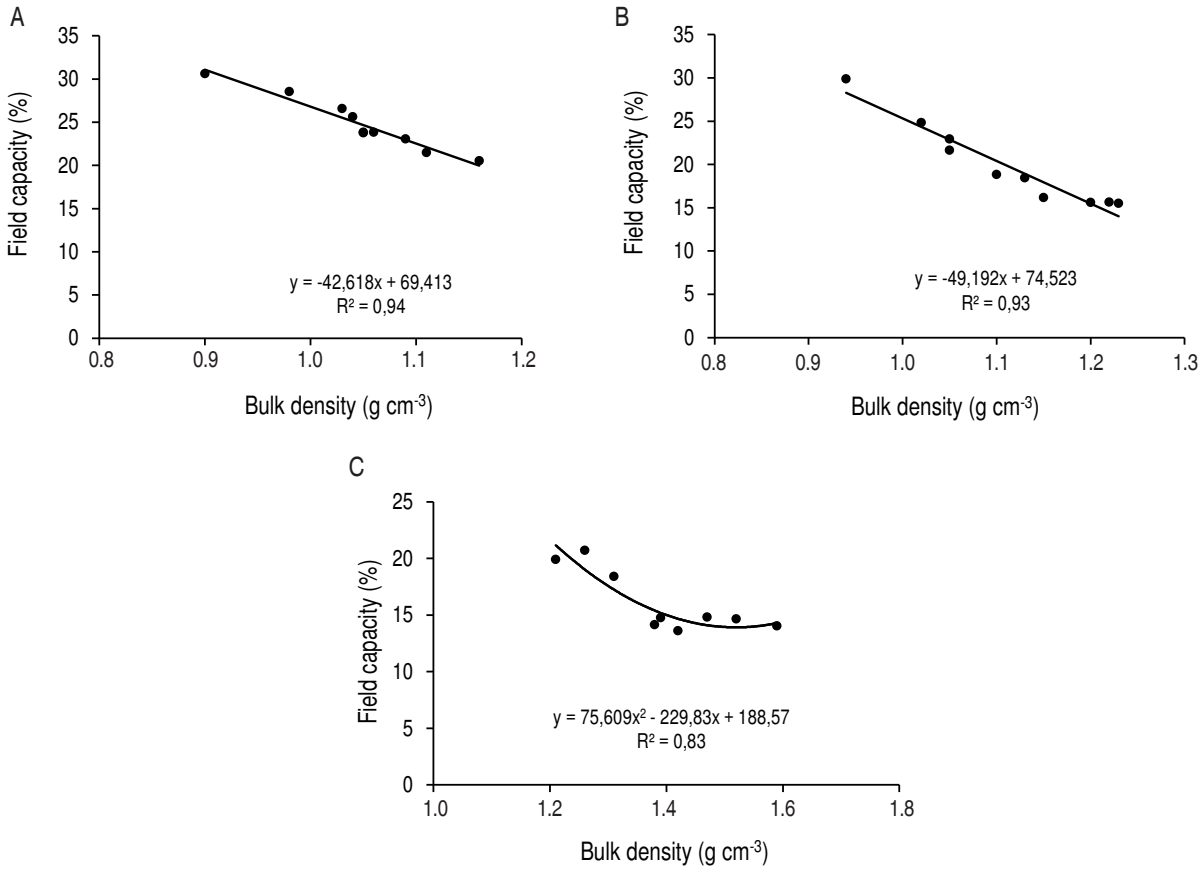

Figure 4. Relationship between field capacity and bulk density: A. Benchmark profile, B. Conservation profile, C. Agrogenic profile.

Table 2. Average soil particle size distribution across three land use types and three depths.

Land use	Depth (cm)	Sand	Silt	Clay	Structural index	OM
		(%)				
Benchmark	0–15	20.43	5.52	62.23	9.30	6.3
	15–25	17.06	3.61	63.82	5.78	3.9
	25–50	15.78	3.87	64.76	4.52	3.1
Conservation	0–15	19.76	3.74	58.38	5.15	3.2
	15–25	17.26	4.51	61.18	5.33	3.5
	25–50	12.79	4.74	66.97	2.09	1.5
Agrogenic	0–15	18.66	8.41	62.45	2.54	1.8
	15–25	16.85	8.53	64.63	1.37	1.0
	25–50	15.21	6.41	65.57	0.83	0.6

Olivera (2017), as soil compaction increases under heavy farm machinery, the soil physical structure deteriorates, bulk density increases and total porosity decreases.

The organic matter content and the structural index shows a linear relationship with high degree of dependence (R^2=0.99). Therefore, with increasing organic matter the structural index increases and vice versa (Figure 5). This relation may be more dependent for this type of soil dominated by oxides and 1: 1 mineral compared to soils dominated by 2: 1 mineral (Six *et al*., 2000). It should be noted that predictions made by using the regression line fitting (R^2=0.99) obtained in this figure produced practically the same results as the regression on individual soil management.

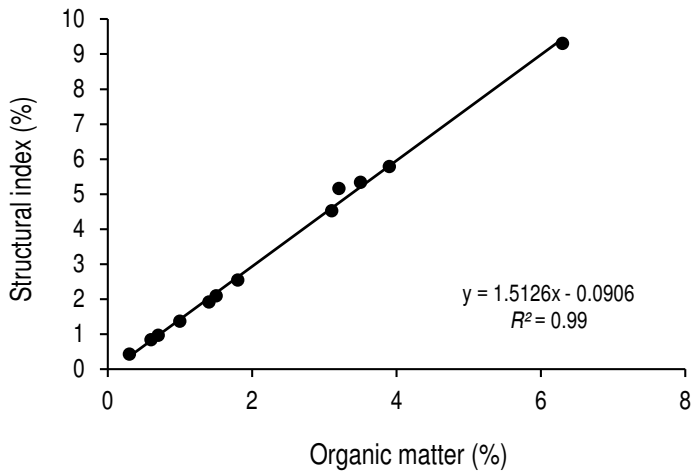

Figure 5. Relationship between organic matter and structural index.

CONCLUSIONS

Our study shows that all soil properties were markedly different between native forest (Benchmark) and the continuous cultivation (Agrogenic). The other land types increased bulk density, decreased field capacity, organic matter, porosity and structural index. Continuous cultivation caused the degradation of the topsoil eventually reaching a 50 cm depth which leads to a greater compaction, porosity limiting and humidity reserve. Benchmark profile did not present significant differences with the conservate profile which we attribute the No-till policy and adequate rest time. Establishing pastures could help reduce bulk density and improve the soil physical properties.

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Bioclimatic analysis of three buildings for wet processing of coffee in Colombia

UNIVERSIDAD NACIONAL DE COLOMBIA

Análisis bioclimático de tres instalaciones para el beneficio húmedo de café en Colombia

doi: 10.15446/rfnam.v71n3.64566

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ABSTRACT

This study aimed to perform a bioclimatic comparison of wet processing facilities of coffee in Colombia, with three typical types of Colombian coffee region, through computer simulation, specifically evaluating the effect of heat and steam generated by mechanical drying machines, and natural ventilation area on the temperature and relative humidity within these facilities. The effect of the natural ventilation area was observed, indicating that the greater the natural ventilation area, the lower the temperature and relative humidity, i.e. it was observed that typology b behaved better bioclimatically than typology a. The indoor environment of type a (stepped type), had a greater biological risk of proliferation of fungi and bacteria, with an average temperature of 27.5 °C, and average internal relative humidity of 70.6%. In type c, as its mechanical drying machine protrudes from the building, and expel the vapor and heat produced in the drying process to the external environment, showed the best bioclimate conditions for parchment coffee, with an average temperature of 23.5 °C and average internal relative humidity of 65.5% most of the time.

Keywords:
Bioclimatic simulation
Biological risk
Quality coffee
Wet processing
Coffee drying

RESUMEN

Este estudio tuvo como objetivo llevar a cabo una comparación bioclimática de instalaciones de beneficio húmedo en Colombia, con tres tipologías típicas de la zona cafetera colombiana, a través de simulación computacional, específicamente evaluando los efectos del calor y vapor generados por el secado mecánico, y el área de ventilación natural sobre la temperatura y la humedad relativa en estas instalaciones. Se observó el efecto del área de ventilación natural, a mayor área de ventilación natural, menor temperatura y humedad relativa, es decir, se observó que la tipología b se comportó mejor bioclimáticamente que la tipología a. El ambiente interno de la tipología a (tipología escalonada), tuvo un mayor riesgo biológico de proliferación de hongos y bacterias, con una temperatura promedio de 27,5 °C, y una humedad relativa interna promedio de 70,6%. En el tipo c, como su secadora mecánica sobresale del edificio, y expulsa el vapor y el calor producidos en el proceso de secado al ambiente externo, mostró las mejores condiciones bioclimáticas para el café pergamino, con una temperatura promedio de 23,5 °C y una humedad relativa interna promedio del 65,5% la mayor parte del tiempo.

Palabras clave:
Simulación bioclimática
Riesgo biológico
Calidad de café
Beneficio húmedo
Secado de café

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The coffee postharvest process is very important for preservation of coffee quality bean (Ribeiro *et al*., 2011; Carvajal *et al.,* 2012). In Colombia, coffee is processed with wet way. The wet process of coffee includes depulping, fermentation, sorting, washing and drying the coffee bean. Coffee is often stored in post-harvest facilities.

Drying is considered a critical step of process. The coffee is dried in the interest of maintaining quality and storing it for extended periods of time (Borém *et al.,* 2007; Ciro *et al.,* 2010; *Ciro et al.,* 2011). The main problems in the cup (taste) arise from poor drying and storage. The development of microorganisms is a factor in the environment that arises only when adverse storage conditions permit excessive moisture accumulation in the grain bulk or when grain is initially stored above permissible safe moisture contents required for its preservation (Puerta, 2008; Oliveros *et al.,* 2013).

As the harvest of coffee occurs in the rainy season in Colombia, some of the time, the external environment has relative humidity above 70%; in the case of natural ventilation, this constitutes a natural bioclimatic limitation (Osorio *et al.,* 2015).

The bioclimatic environment plays an important role in the conservation of parchment coffee quality. Fungi live and reproduce best in a range of 70 to 80% relative humidity, whereas yeast and bacterial development require humidity higher than 85% in the intergranular air; temperatures higher than 50 °C can kill the embryos from coffee seeds and start the decomposition process. In the tropical zones with relative humidity above 70%, and temperatures above 27 °C, freshly harvested grains, are in favorable environment to the development of insects (Navarro and Noyes, 2001).

The biological risk is latent in facilities that perform the wet processing of coffee; for example, according to Puerta (2006), *Aspergillus ochraceus* (the leading producer of Ochratoxin A) was found in 70% of the facilities tested in Colombia, in coffee postharvest facilities, as well as solar dryers, parchment coffee and green coffee. This author argued that when adequate conditions of humidity, temperature, time and poor hygiene occur, these microorganisms can proliferate.

Approximately, 70% of the volume of coffee that is produced in Colombia is dried mechanically (González *et al.,* 2010), generally inside the wet processing facilities of coffee. In Colombia, this process usually generates a lot of steam and heat in the facilities where the coffee is dried and stored. In post-harvest process, the largest power consumption occurs in the mechanical drying process, which, having an efficiency of 50% (Puerta, 2006), added large amounts of steam and thermal energy to the building, which increases the temperature and moisture inside. Consequently, the biological risk is increased.

Poor control and design of buildings for the wet processing of coffee can compromise product quality due to inadequate bioclimatic environments (Osorio *et al.,* 2015). Humid environments and high temperatures during storage are risk conditions that can physically damage the grain, causing decomposition and deterioration of the quality of the product (Puerta, 2008). To analyze and suggest bioclimatic and air quality solutions within agro-industrial buildings, the application of mathematical and computational modeling and simulations is increasingly used (Norton *et al.,* 2009). Simulation is a very interesting tool in the design and evaluation of buildings, as the bioclimatic conditions of the buildings involve complex aspects such as energy flows, transient weather variables, stochastic occupancy patterns, etc., that traditional design methods based on experience or experimentation cannot satisfactorily quantify (Bre *et al.,* 2013).

EnergyPlus™ is one of the most used programs for energy and bioclimatic simulation for buildings (DoE, 2012), which is a free open source software developed by the US Department of Energy (DoE). This program was used by Osorio *et al.* (2015) and Osorio *et al.* (2016) for the bioclimatic and energy analysis of coffee post-harvest facilities with good results.

Its main input variables are: 3D building design, physical and thermodynamic properties of building materials, internal equipment, and weather file of the site where the building is located, in order to perform transient analysis for energy efficiency, bioclimatic variables and air quality within buildings, across balances of mass, energy and chemical composition.

This study aimed to simulate the thermal environment of three typical installations of the Colombian coffee postharvest, in order to compare and analyze the internal bioclimatic conditions, in terms of temperature and relative humidity in order to preserve grain quality.

MATERIALS AND METHODS

Location of buildings and production

The three buildings are located in the department of Antioquia – Colombia, in the municipality of Barbosa (at coordinates 6°26'15"N, 75°19'50"W), near to Medellin city. For these simulations, the climate file of Medellin was used (at coordinates 6°14'41"N 75°34'29"W, altitude of 1500 m), which has a representative climate for coffee of this zone (average conditions of temperature of 16 to 28 °C and relative humidity of 60 to 80%). The three farms produce the same amount of coffee, with a coffee cherry production of about 156,250 kg per year^{-1} (31,250 kg per year of parchment coffee). This study was conducted during the month of November (main harvest of 2015).

Description of the buildings

The facilities volumes 3D were drawn in SketchUp® program (Figure 1). The first geometry, type a (Figure 1A), has two floors (of equal size) in a stepped form. On the first floor was the mechanical drying area, while the second floor contained the area for pulped and fermented coffee. The dimensions of this building are: 5.50 m wide x 9.50 m long x 4.0 m high.

Type b (Figure 1B) has two separate floors: the first floor is the area for pulping, fermentation and mechanical drying of the coffee and has dimensions of 11.0 m long x 6.0 m wide x 3.5 m high. The second floor consists of a parabolic solar dryer with plastic covering, measuring 11.0 m long x 6.0 m wide x 2.3 m high. Between the first and second floor, there is a lightweight concrete and brick slab.

Type c (Figure 1C) has two rooms: the main room is the area for the pulping and fermentation of coffee, while the second room is a mechanical dryer for the coffee. Type c has dimensions of 10.40 m long x 5.0 m x wide x 2.7 m high. The second room has dimensions of 2.0 m long x 2.0 m wide x 4.60 m high. Unlike types a, and b, in the type c, as its mechanical drying machine protrudes of the building, expels the vapor and heat produced in the drying process to the external environment.

Each floor of type b, as well as the room for the wet processing of coffee and mechanical drying machine of type c, were analyzed as an independent thermal area, while type a was analyzed as a single thermal zone. In each thermal zone, the thermal characteristics of the materials and other details of each patterned surface are described (DoE, 2014).

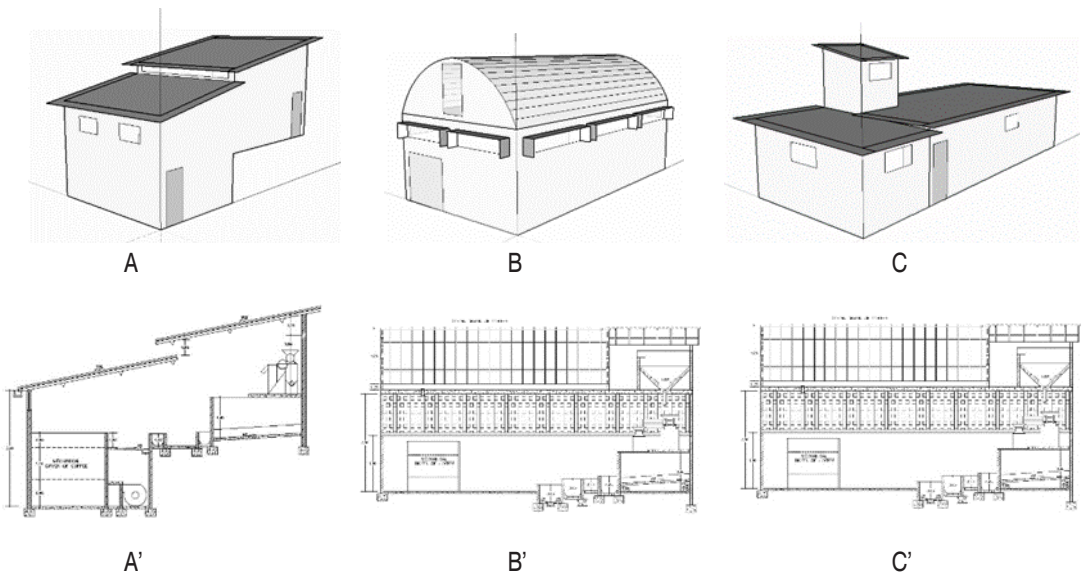

Figure 1. 3D geometries and architectural cuts of three buildings for the wet processing of coffee types a (A, A'), b (B, B') and c (C, C').

The three types were built with 15 cm unplastered brick, and the windows of all buildings were open all the time. Types a, and c had a fiber cement roof, whereas type b had a lightened slab between the first floor and the solar dryer (second floor).

In the three types, there were concrete stairs, fermentation tanks, and a coffee hydraulic classifier inside; to these models, one layer of concrete and other of ceramic were added at the bottom, with a volume of 3.5 m^3 in order to account for the effect of thermal inertia of this mass. In addition, it were measured the volume and natural ventilation area of each building of each type.

Boundary conditions

The second floor of type b corresponds to the parabolic solar dryer of the coffee, which has a polyethylene plastic cover and a coffee layer with an average humidity of 33% wb and a 0.03 m thick slab. The solar dryer has two openings of 1.6 m^2 each for natural ventilation during the day. For the night conditions, it is assumed that the solar dryer was closed. With respect to equipment, for all three types, there are lights, a humidity processing module to peel and sort coffee with a capacity of 2000 kg of coffee cherry per hour, and a mechanical drying machine with a capacity of 1125 kg of parchment coffee per day.

In the contour conditions of the models, for the thermal properties of the washing coffee, we used equations 1 and 2 proposed by Montoya *et al.* (1990), for the specific heat C_p, J kg^{-1} $°K^{-1}$) and density of the coffee (ρ_C, kg m^{-3}). These properties are functions of the moisture content of the product on a dry basis (M_{db}, decimal dry basis).

$$C_p = 1.3556 + 5.7859M_{db} \quad (1)$$

$$\rho_C = 365.884 + 2.7067M_{db} \quad (2)$$

The thermal properties of building materials used as boundary conditions is shown in Table 1.

Table 1. Thermal properties of construction materials.

Material	λ (W m^{-1} $°K^{-1}$)	ρ_C(kg m^{-3})	C_p(kJ kg^{-1} $°K^{-1}$)
Brick	1.05	1800	0.92
Ceramics	0.65	1600	0.84
Concrete	1.75	2200	1.00
Fiber cement	0.65	1600	0.84
Mortar	1.15	2000	1.00
Polyethylene plastic	0.40	920	1.90
Steel - Iron	55.00	7800	0.46

ρ: density, λ: thermal conductivity, C_p: specific heat.
Source: Adapted from NBR-15220 (ABNT, 2003), INMETRO, (2013),and LabEEE, (2015).

For the boundary conditions of energy balance, it is necessary to calculate the heat generated within each building (heat generated by machines, luminaries and human metabolism). Table 2 shows the power values of the machines and lighting. For the three types, simulations were performed with heat exchanger drying machines using coal (anthracite) as fuel, which, according to Oliveros *et al.* (2009) has a consumption of 0.224 kg of coal per kg of dry parchment coffee, with a calorific value of 33440 kJ kg^{-1}.

Table 2. Power of coffee processing equipment.

Equipment	Power	Unit
Engine of depulping module	3357	W
Engine of fan of mechanical dryer	2238	W
Heat exchanger of mechanical dryer	130044	W
Luminaires	10	W m^{-2}

The metabolic rate, i.e., the metabolic energy that the human body expends while performing physical activities, varies from person to person, according to the activity and working conditions performed. The value per person of 423 W was used in this study for the metabolic rate as, according to ASHRAE (2001); this value corresponds to heavy work activity and the handling of 50 kg sacks.

Table 3 shows the usage patterns of the three wet coffee processing facilities models, that is, when the machines are operating, and how many workers are into facilities and in that schedule. Mechanical drying and pulping require the same working hours for three typologies. The solar dryer (in type b) is only opened during the day to encourage the mass exchange of water, and is closed at night to retain thermal energy, prevent condensation and prevent the ingress of moist air from outdoors.

The internal environment of the buildings was simulated for the month of November, during the main harvest of the year, which coincided with the second season rainfall in this part of Colombia (Oviedo and Torres, 2014).

Table 3. Usage patterns in the facilities of wet processing of coffee.

Hours	L	MD	O	PC	SD
00:00-06:00	0	0	0	0	0
06:00-07:00	1	1	2	0	0
07:00-07:30	0	1	0	0	1
07:30-09:00	0	1	0	0	1
09:00-10:00	0	1	1	0	1
10:00-10:30	0	1	0	0	1
10:30-12:00	0	1	0	0	1
12:00-13:00	0	1	2	1	1
13:00-13:30	0	1	0	0	1
13:30-14:00	0	1	0	0	1
14:00-15:00	0	1	0	0	1
15:00-15:30	0	1	1	0	1
15:30-16:00	0	1	1	0	1
16:00-17:30	0	1	0	0	1
17:30-18:00	1	1	0	1	1
18:00-19:00	1	1	1	1	0
19:00-20:00	1	1	2	0	0
20:00-21:00	1	1	2	0	0
21:00-24:00	0	0	0	0	0

L: luminaires, MD: mechanical drying, O: occupants, PC: pulping coffee, SD: solar drying.

Statistical analysis

A statistical analysis of variance (P<0.001) and test media (Tukey, P<0.05) was performed for the analysis of temperature and relative humidity (hourly), with four treatments: outdoor, type a, b, c and outdoor as a control.

In addition, an analysis of the number percentage of hours that facilities remained with a relative humidity between 90 and 100%, 70 and 90% and less than 70% was performed, in order to assess the bioclimatic environment and biological risk for the preservation of the parchment coffee quality .

RESULTS AND DISCUSSION

Comparing type a, and b, the effect of natural ventilation area on lowering internal temperature was observed, agreeing with Osorio *et al*. (2015), and Osorio *et al*.

(2016). Nevertheless Table 4 shows that the type c building although had the smallest ventilation area, the steam and heat of the mechanical drying process is removed from the building, as its mechanical drying machine protrudes from the building, to expel the vapor and heat produced in the drying process to the external environment. Its average temperature and relative humidity were statistically lower (Table 5).

Table 4. Volume built and natural ventilation area.

GN	V (m^3)	VA (m^2)	VA/V ($m^2 m^{-3}$)
Type a	209	2.82	0.0135
Type b	231	17.40	0.0753
Type c	140	1.44	0.0103

GN: Group name, V: Volume, VA: Ventilation area.

On the other hand, type a, presented an internal environment with an average temperature higher than above 27 ºC and average relative humidity above 70%, which according to Navarro and Noyes (2001), constitutes an environment of high risk of attack of fungi and bacteria in grain storage; this means that type a, presented the higher biohazard in its indoor environment, due to its high average temperature and high relative humidity.

Table 5. Statistical data of mean temperature.

Group name	N	Temperature		Relative humidity	
		Mean	Std Dev	Mean	Std Dev
Outdoor	720	21.88 a	2.96	69.3 a	14.5
Type a	720	27.46 b	7.49	70.5 a	15.4
Type b	720	24.96 c	4.11	66.7 b	13.6
Type c	720	23.55 d	3.56	63.4 c	9.7

Means followed by the same letters do not differ by the Tukey test at 0.05 probability (P<0.001, F=165.94 for temperature analysis, F=30.43 for relative humidity analysis).

Taking into account that the grain moisture contents that are in equilibrium with the surrounding air containing a lower relative humidity than 70%, are considered safe (Navarro and Noyes, 2001; Puerta, 2008), and the internal environment of type a, was close to saturation 9.70% of the time, and spent more than half of the time with a relative humidity greater than 70%, 51.52% of the time (Table 6), this leads to a danger of proliferation of fungi and bacteria that are detrimental to grain quality.

On the other hand, type c, presented the most adequate bioclimatic conditions to conserve the quality of dried parchment coffee (under storage conditions), since in the month of harvest peak and rainiest in the study area (November) the Relative humidity was in a safe range 70.64% of the time, with an average relative humidity of 63.4% and an average temperature of 23.5 °C, decreasing the risk of attack by fungi, bacteria and insects (Navarro and Noyes, 2001; Puerta, 2008).

Table 6. Percentage of time remaining at different relative humidity.

Group name	N	Percentage of time in the range of relative humidity		
		90-100%	70-90%	<70%
Type a	720	9.70	41.83	48.48
Type b	720	5.68	38.23	56.09
Type c	720	0.42	28.95	70.64

In Colombia, it is common to store dry parchment coffee during the harvest in buildings of processing of coffee for several weeks, in order to increase the volume of dry parchment coffee to transport and save money on freight costs (Osorio *et al*., 2015). In this context, type a, had a greater biological risk regarding the creation of fungi, bacteria and insects (hot and humid environment), meaning that the grain may be re-moistened and damaged (Puerta, 2008). In the same context, type c showed the most innocuous bioclimate (safe biologically), with a lower average temperature of 27 °C (Table 5), an internal relative humidity less than 70% most of the time, with a humidity close to saturation for almost no time (Table 6).

CONCLUSIONS

In the buildings of post-harvest coffee with steam and heat generation inside, the effect of ventilation on lowering internal temperature was observed. However, in this study was observed that the best bioclimatic results for coffee parchment were presented in the case where mechanical drying steam from coffee was thrown directly into the external environment (type c).

The indoor environment of type a (average temperature above 27 °C, and average humidity above 70%), had a greater biological risk of the creation of fungi and bacteria, meaning that the grain may be re-moistened and damaged. In type c, as its mechanical drying machine protrudes from the building, to expel the vapor and heat produced in the drying process to the external environment, this type showed the most innocuous bioclimate conditions (safe biologically), with average temperature of 23.5 °C and average relative humidity 63.4% relative humidity.

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Effect of drying methods on physical and chemical properties of *Ilex guayusa* leaves

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Efecto de métodos de secado sobre las propiedades físicas y químicas de las hojas de *Ilex guayusa*

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ABSTRACT

The influence of air-drying (AD), convection oven (CO) and solar drying (SOD) on the physical and chemical properties of *Ilex guayusa* leaves is discussed. Total ash (%), acid-insoluble ash (%), water-soluble ash (%), residual moisture (%) and caffeine content (%) were estimated. Additionally, alkaloids, flavonoids, reducing sugars, saponins, steroids, quinones, fats, phenols and tannins detection of dry leaves extracts were assessed. The results revealed that parameters of physical analysis were not affected by the drying techniques. The highest amount of secondary metabolites in ethanol and aqueous extracts were detected. In general, convection oven dried leaves showed the highest caffeine content (3.71%) and the lowest drying time (13 h) compared to other drying methods. The results revealed a fast method to dry *Ilex guayusa* leaves and indicate that this species possess a variety of bioactive compounds beneficial for health. Our results revealed an effective quick method to dry *Ilex guayusa* leaves without altering the content of beneficial bioactive components.

Keywords:
Ilex guayusa
Caffeine
Air drying
Conventional oven
Solar drying

RESUMEN

En este trabajo se discute la influencia del secado al aire (AD), el horno de convección (CO) y el secado solar (SOD) sobre las propiedades físicas y químicas de las hojas de *Ilex guayusa*. Se estimaron las cenizas totales (%), las cenizas insolubles en ácido (%), las cenizas solubles en agua (%), la humedad residual (%) y el contenido de cafeína (%). Además, se analizó la presencia los alcaloides, flavonoides, azúcares reductores, saponinas, esteroides, quinonas, grasas, fenoles y taninos en extractos de hojas secas. Los resultados revelaron que los parámetros del análisis físico no se vieron afectados por las técnicas de secado. La mayor cantidad de metabolitos secundarios se detectó en el extracto etanólico y acuoso. En general, las hojas secadas al horno de convección mostraron el mayor contenido de cafeína (3,71%) y el menor tiempo de secado (13 h) en comparación con los otros métodos de secado empleados. Los resultados obtenidos revelaron un método rápido para secar hojas de *Ilex guayusa* sin alterar el contenido de componentes bioactivos beneficiosos para la salud, estudios que no han sido reportados.

Palabras clave:
Ilex guayusa
Cafeína
Secado al aire
Horno convencional
Secado solar

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Guayusa is the common name for *Ilex guayusa* which is a wild tree that belongs to the family Aquifoliaceae (Radice *et al.,* 2017). It is a holly species found in Colombia, Ecuador and Perú (Dueñas *et al.,* 2016). Decoctions of the leaves are consumed as a stimulating morning drink (Tene *et al*., 2007). Amazonian Kichwa people prepare infusions every day to promote conviviality, for festivals and rituals (Overing *et al.,* 2000). On the other hand, mestizos and white populations in Ecuador consume the infusion as an additive to spirits (Dueñas *et al.,* 2016).

The main compounds reported in the leaves and tea of *I. guayusa* are caffeine, theobromine and polyphenols (Radice *et al*., 2017); chlorogenic acid, lutein and quercetin-3-O-hexose (Villacís-Chiriboga *et al*., 2017); phenolic mono- and dicaffeoylquinic acid derivatives (Pardau *et al.,* 2017). In addition, these authors report guayusa leaves have antioxidant and anti-inflammatory activity. Caffeine (1,3,7-trimethylxanthine) is an alkaloid of great interest because of the beneficial effects that brings to individuals. For instance, it increases alertness, improves concentration and exhibits antioxidant properties (Turnbull *et al.,* 2017; Metro *et al*., 2017). Thus, to quantify the content of caffeine in the leaves of *I. guayusa*, it is important to determinate the quality of the final product.

The Ecuadorian guayusa has a great demand in the international market for its good sensory attributes assigned to the preparations obtained from the leaves of the species. Ecuador exports almost 100% of the harvest which is reduced 75% to dry of the total fresh leaves collected (Proecuador, 2017)[1].

The producers commercialize aromatic and medicinal herbs by putting them through a drying processes. This is a complementary way of selling and inserting guayusa into new markets. The drying method is a critical step in the process because it extends the shelf life of herbal products (Figiel *et al*., 2016). This process inhibits microbial growth and prevents biochemical changes. Thus, it minimizes microbial contamination and possible modifications from the physical and chemical point of view and increases the shelf life of the product (García, 2014).

[1] https://www.proecuador.gob.ec/ficha-de-guayusa/

The most common methods are air-drying (AD), convection oven (CO) and solar drying (SOD). AD is very simple and economical, but it takes long periods to reach the adequate moisture content and may increase the probability of microbial spoilage (Kim *et al*., 2012). CO is considered as a cost – effective method but some compounds can get lost as a consequence of the high temperature (García, 2014). However, we have not found studies about the drying methods used to improve the stability of the leaves of *I. guayusa*.

The objective of this research was to determine the effect of air-drying, convection oven and solar drying on the physical and chemical properties of the leaves of *Ilex guayusa.*

MATERIALS AND METHODS

Plant materials and reagents

Fresh leaves of *I. guayusa* in vegetative phenological state were collected in *S*ucumbíos, Ecuador. A sample of the plant material was taken for botanical identification at National Herbarium of Ecuador, keeping an herbal witness (CIBE 020) at Centro de Investigaciones Biotecnológicas del Ecuador (Guayaquil – Ecuador). The plant material was previously washed and selected before drying process. After that, the dried material was grinded to homogeneous powder (800 µm) previous to physical and chemical analysis. All the chemicals were of analytical grade. The water was purified in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Drying methods

Air Drying (AD). Leaves of *Ilex guayusa* were air-dried at ambient temperature in a ventilated room without exposure to solar light. The highest temperature recorded was 31.83 °C and the lowest was 21.94 °C. The loss weight was measured every 24 h for 13 days until it reached a constant weight.

Convection Oven (CO). The experiments were performed at 46 °C in a convection oven (VWR Scientific Products, Atlanta, USA). Leaves were distributed uniformly on perforated stainless-steel trays (0.76 x 0.51 m). The time required to reach a constant weight of dried leaves was 13 h (Díaz-Maroto *et al.,* 2003).

Solar Drying (SOD). The plant material was dried in a handmade chamber solar dryer equipped with a forced

air circulation system through an average temperature of 33.54 °C. Samples were placed uniformly on perforated stainless-steel trays (0.50 x 0.50 m) into the system. The time required to reach a constant weight of dried leaves was 36 h.

Physical and chemical properties

Weight loss for fresh samples were obtained by the three different drying methods until constant mass was reached. Moisture was determined from sample weight loss after oven drying at 105 °C for 3 h (AOAC, 2005; method 930.15). Total ash, acid-insoluble ash and water-soluble ash were determinate for all samples (WHO, 1998).

Phytochemical analysis

Qualitative phytochemical analysis was performed using ethyl ether, ethanol and water solvents according to the procedure described by Miranda and Cuellar (2001). Briefly, 30 g of sample was weighted and 300 mL of solvent of increasing polarity was added, each one after 24 h of successive maceration extraction at temperature room. The presence or not of the following chemical compounds was determined: oils - fats; alkaloids; flavonoids; quinones; saponins; lactones; phenol and tannins; reducing sugars; mucilages; bitter principles; steroid and triterpenoid; aminoacids; resins.

Determination of caffeine

Grinded guayusa (500 mg) was added into 25 mL of distilled water at boiling temperature. Then, it remained without further heating for 10 min. The extracts passed through a filter paper (Whatman number 43), and then filtered through 0.22 µm disposable filters before analysis (Cai *et al*., 2004; Lima *et al*., 2004; Venditti *et al*., 2010). Five working solutions of standard caffeine were prepared in the range of 10 to 100 mg L^{-1} to determine the percentage of caffeine in aqueous solution. From the calibration curve, the correlation coefficient was r^2=0.998.

Chromatographic analysis was performed in a high-performance liquid chromatography (HPLC) with diode array detector system (Perkin Elmer, Norwalk, CA, USA) equipped with a C18 250 mm x 4 mm (5 µm). The mobile phase consisted of a mixture of acetonitrile and water (10:90, v/v) at a flow rate of 1.0 mL min^{-1}. Chromatograms were measured at 254 nm with a run time of 5 min (Rojo *et al*., 1999).

Statistical analysis

All assays were performed at least in triplicate. Analysis of variance (ANOVA) was employed to determinate significant difference between samples using the software Statgraphics Centurion XVI (Statistical Graphics Corporation, Rockville, USA).

RESULTS AND DISCUSSION

Physical and chemical properties

The observed values of total ash, ash insoluble in acid and soluble in water, showed no significant differences depending on the drying methods used (Table 1). This result was expected since mineral elements have a high stability. Further, minerals in the soil represent the ashes. The plant absorbs them through the roots. Hence, depending on the place of collection and the type of soil, ashes can vary, both in concentration and in qualitative composition.

The total content of ashes found (6.8%) was higher than that reported by Kapp *et al*. (2016) (4.9%) for guayusa leaves. It is high for the ranges established in the pharmacopoeias and the norms that determine a total index of ashes up to 5%. However, this value might relate to the concentration of minerals in the soil. As observed in the percentage of water-soluble ash, they are basically constituted by alkaline and alkaline-earth minerals. Furthermore, the percentage of ash insoluble in acid, representative of the presence of heavy metals, is within the established limits (Zhi-cen, 1980; WHO, 2011).

Moisture is the water content that remains inside the cell, once the extracellular water clears away by drying. Thus, moisture is of great importance because, in high amounts, it indicates a poor drying process and can cause deterioration of the material plant growth by microorganisms and biotransformation of secondary metabolites. The pharmacopoeias refer values of 8 and 14% depending on the vegetable organ studied.

The results of the physical analysis showed moisture values close to 8%, without significant differences between the different types of drying, which means that regardless of the method and the time used, a good drying of the plant material was achieved.

Another aspect analyzed was the concentration of caffeine (expressed in percentage). Hence, a small increase in

concentration was observed in the CO method (3.71% ± 0.11). This may be related to a shorter drying time (13 h CO) compared to air drying (13 d AD) and solar drying (36 h SOD), avoiding the fermentation or degradation of the active ingredients (Christensen and Kaufmann, 1974; Reis *et al*., 2003). The content of caffeine found in guayusa is comparable to values reported for *Camellia sinensis* (Nishitani and Sagesaka, 2004) and higher than the content described for *Ilex paraguariensis* (1.8%) (Isolabella *et al*., 2010).

Table 1. Physical and chemical properties of *Ilex guayusa* leaves dried by different methods.

Parameter	Drying method		
	AD	CO	SOD
Total ash (%)	6.87 ± 0.12 a*	6.85 ± 0.11 a	6.86 ± 0.11 a
Acid-insoluble ash (%)	0.87 ± 0.07 a	0.86 ± 0.03 a	0.87 ± 0.06 a
Water-soluble ash (%)	3.10 ± 2.12 a	3.11 ± 2.12 a	3.09 ± 2.10 a
Moisture (residual) (%)	7.82 ± 0.41 a	7.33 ± 0.44 a	7.65 ± 0.08 a
Caffeine (%)	3.02 ± 0.07 a	3.71 ± 0.11 b	2.98 ± 0.15 a

*Significance letters in the same row indicate significant differences.

Phytochemical analysis

Phytochemical screening is an important test for the identification of a new source of therapeutic compounds from medicinal plants (Ambasta *et al*., 1986). In this study, the identification of metabolites of *Ilex guayusa* leaves dried by different methods were performed at ethyl ether (EEE), ethanol (EE) and aqueous extracts (AE). Plant constituents such as alkaloids, flavonoids, phenols, tannin, reducing sugars were identified at EE and AE. Meanwhile steroid, triterpenoid and quinones were only found at EE and saponins at AE (Table 2).

Table 2. Phytochemical evaluation of *Ilex guayusa* leaves dried by different methods.

Metabolite	Method	Extraction solvent								
		Ethyl ether (EEE)			Ethanol (EE)			Aqueous (AE)		
		SOD	AD	CO	SOD	AD	CO	SOD	AD	CO
Alkaloids	Dragendorff	-	-	-	-	-	-	+++	+++	+++
	Wagner	-	-	+	+	+	+++	++	++	
	Mayer	-	-	-	+	+	+	+	+	+
Flavonoids	Shinoda			-	-	-	+	+	+	
	Anthocyanins				-	-	-			
	Catechins				+	+	+			
Phenols and tannin	Ferric Chloride			+	+	+	+	+	+	
Reducing compounds	Fehling			+	+	+	+	+	+	
Mucilages	Mucilages							-	-	-
Bitter principles	Organoleptic							-	-	-
Saponins	Foam				-	-	-	+	+	+
Steroid and triterpenoid	Liberman Buchard	+	+	+	+	+	+			
Aminoacids	Ninhidrina					-				
Quinones	Borntranger				++	++	++			
Oils and fats	Sudan	+	+	+						
Lactones	Baljet	-	-	-	-	-	-			
Resins	Resins				-	-	-			

Air-drying (AD), convection oven (CO), solar drying (SOD). +: presence, –: absent

These results differ in the diversity of groups with those reported by Pacha (2012), which only indicated the presence of alkaloids and reducing compounds in the AE. However, they coincide in diversity with the work of Kothiyal *et al*. (2012), who report the presence in the genus of different pentacyclic triterpenoids derived from α and β-amyrins, saponins, flavonoids and purine alkaloids, among others. In addition, Villacís-Chiriboga *et al*. (2017) identified and quantified a total of 14 phenolic compounds in the leaves where chlorogenic acid and quercetin-3-O hexose were the major. Also, seven carotenoids were detected and lutein was the main component. The presence of alkaloids in the extracts could be explained by the caffeine content (purine alkaloid) of the leaves which has been reported by Verpoorte *et al*. (2007). Moreover, the different drying methods did not affect the presence of the compound in the different extracts.

CONCLUSIONS

The sample dried in a conventional oven showed a slight increase in the concentration of caffeine in comparison with the other drying methods (AD and SOD). Although, AD and SOD methods did not affect the caffeine concentration. The physical properties such as moisture, total ash, water soluble ash and acid insoluble ash, did not differ significantly for any method. The chemical groups found in all the samples were flavonoids, phenols, tannins, reducing compounds, saponins, triterpenes-steroids, quinones, fats and alkaloids.

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Satisfaction among dairy farm owners after certification on good management practices

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Satisfacción entre propietarios de granjas lecheras luego de certificarse en buenas prácticas ganaderas

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ABSTRACT

The adoption of good management practices (GMP) in dairy farming implies changes that generate satisfaction for the personnel involved in the milk production process. The objective of this study was to assess the level of satisfaction obtained among GMP-certified producers. A total of 37 farm owners in northern Antioquia were surveyed. Data were analyzed using factor analysis and structural equations. A "General satisfaction" variable was analyzed using recursive partitioning and regression trees. Producers were satisfied with the GMP certification, mostly with the changes achieved in working conditions, interrelationships and staff training. The GMP satisfy owners by generating positive changes in the working conditions of the personnel, providing permanent training, and improving their image to other farmers and milk-buying companies.

Keywords:
Dairy cattle
Personnel satisfaction
Social behavior
GMP certification

RESUMEN

La adopción de buenas prácticas ganaderas (BPG) en producción de leche implica cambios generadores de satisfacción al personal involucrado en el proceso productivo. Con el objetivo de evidenciar la satisfacción de productores certificados, se encuestaron 37 propietarios de predios lecheros del norte antioqueño, los datos fueron analizados mediante análisis factorial y ecuaciones estructurales. La variable "satisfacción general" se analizó mediante partición recursiva y árboles de regresión. Los productores están satisfechos con la certificación principalmente con los cambios en las condiciones laborales, las interrelaciones y la capacitación del personal. Las BPG satisfacen a los propietarios de sistemas lecheros, al generar cambios en las condiciones laborales del personal, propiciar capacitación permanente y mejorar su imagen ante productores y empresas compradoras de leche.

Palabras clave:
Ganado de leche
Satisfacción personal
Conducta social
BPG certificación

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Milk is regarded a complete food because it contains protein, fat, carbohydrates, vitamins and minerals (Agudelo and Bedoya, 2005; Claeys *et al*., 2014; De Silva *et al*., 2016) which benefit the health of the consumer (Yuen *et al*., 2012). According to Tafur and Nieto (2011), Battaglini *et al*. (2013) and Cerón-Muñoz *et al*. (2015), milk production requires animal management, sanitary and nutritional practices that guarantee a safe product (Young *et al*., 2010) through the application of good management practices (GMP). Tafur and Nieto (2011) defined GMP as a tool to monitor routinely activities involved in dairy production, while Young *et al*. (2010) defined them as a collection of management and biosecurity measures to prevent the risk of milk contamination with chemicals or physical elements or infection of animals by microorganisms. According to Londoño *et al*. (2016), GMP are those practices directed to the continuous improvement of processes in a livestock system, aimed at promoting business management while ensuring a safe product.

Colombian decree No. 616/2006 regulates milk production in the country by ordering a series of measures to standardize the hygiene and sanitary parameters of raw milk (MPS, 2006). In chapter 63 the need to create a system that supervises and certifies the safety of raw milk is exposed, this is how GMP arose. It also grants power to the Colombian Agricultural Institute (ICA) to carry out the inspection, evaluation and official certification of dairy farms, officializing GMP application (ICA, 2008). Decree 0017/2012 (MADR, 2012) and amendments 0077 (MADR, 2015) and 468 (MADR, 2015) establish the payment methodology to the farmer and define GMP as the payment criterion.

The application and compliance of GMP implies that milk producers have to invest time and money in the certification process. In return, they expect satisfactory results for applying GMP. Satisfaction is defined as compliance with requirement, or as being content and in accordance with what is done. Veenhoven (1996) goes beyond the cognitive definition by stating that a satisfied individual is one who enjoys life; while Maslow (1943) argued that satisfaction is achieved by solving specific needs.

The study of satisfaction has been approached by Herzberg, who proposed the theory of factors, indicating that satisfaction depends on the fulfillment of all the factors involved in the work environment (Pinto, 2002). David McClelland presented the theory of learned needs, which defines the attitudes of an individual with respect to their state of satisfaction (Perilla, 1998). Maslow (1943) proposed the theory of pyramidal hierarchy of human needs in which the most basic needs are on the basis, while security, appreciation and esteem needs are above and self-realization at the apex.

The objective of this study was to measure the degree of satisfaction of dairy farmers with certification in good management practices.

MATERIALS AND METHODS

The survey was applied to GMP-certified dairy farm owners in northern Antioquia (municipalities: Bello, San Pedro de los Milagros, Entrerríos, Don Matías, Santa Rosa de Osos, San José de la Montaña, Belmira and Yarumal).

The Likert-scale questionnaire was written considering the hierarchy of needs theory (Maslow, 1943). The dimensions considered the achievements reached by producers when they got the GMP certificate, responding to the needs of security, esteem and self-realization (8 variables, Table 1). The second dimension measured the level of satisfaction with the changes obtained (9 variables, Table 1). The third dimension evaluated how easy the certification requirements were (5 variables, Table 1). Finally, the general satisfaction variable was expressed within the statistical analysis as “pleased”.

A pilot test was applied to five certified producers in order to validate the questionnaire. It evaluated time invested, pertinence of the language, clarity of the questions and extension of the questionnaire.

Once validated, the questionnaire was applied to 37 GMP-certified producers by sampling at the region convenience. Their participation was voluntary and confidential. The Cronbach test (1951) was applied to each dimension to measure reliability of the measuring scales using sjstats (Lüdecke, 2016) in R-project software (R Core Time, 2016), and the criterion was that it exceeded 0.70.

For the statistical analysis, the relationships between variables generated for measuring general satisfaction were evaluated with a model of structural equations by means of

exploratory factor analysis using psych package (Revelle, 2016) in R, choosing those variables that explained more than 65% of the factors.

Once variables with the greatest weight to explain producer satisfaction were obtained, a confirmatory factor analysis was performed including "General satisfaction" as exogenous variable using Lavaan package (Rosseel, 2012) in R. Reliability of the comparative goodness of fit (CFI) was verified between 0.95 and 0.98 (Bentler, 1990), and the square root of the residual mean square residual error (RMSEA) was less than 0.1 (Rigdon, 1996).

The "General satisfaction" variable was also analyzed by recursive participation and regression trees of all satisfaction, frequency and opinion variables applying the rpart package (Therneau *et al*., 2015) in R. The cross validation method (Therneau *et al*., 2015) was used to certify the recursive participation models identifying the optimal complexity parameter (PC) in the pruning of each decision tree.

RESULTS AND DISCUSSION

More than 80% of farmers were older than 30 years of age, implying a high level of maturity, reflected in the decision making that affect farming processes. Regarding schooling, 10 producers earned undergraduate and graduate degrees. According to Nahuelhual *et al*. (2009), the adoption of good practices in milk production has a positive relationship with age and level of education. The producers were all dairy farms owners, with 27 of them also performing farm management functions, implying that their decisions are motivated on productivity and personal interest.

Regarding the first group of variables, producers pointed out that GMP certification allowed them to develop new skills and acquire new knowledge (Table 1). They emphasized that they were happy to reach the proposed goals, which indicates a sense of belonging. According to Maslow's theory (1943), this implies satisfaction of self-realization needs located at the apex of the pyramid explaining hierarchy of needs.

Interestingly, producers feel their image before milk-buying companies and fellow producers improve for applying GMP. According to Nahuelhual *et al*. (2009), the probability of adopting GMP has a positive relationship with the image of the producer.

Producers were satisfied with the changes generated in the farm, both in administration and management practices, resulting from the application of GMP (Table 1). According to Bewley *et al*. (2001), as dairy farmers perceived productive and managerial improvements resulting from applying technology, they felt more satisfied with their daily work and personal life, which was favored by time savings.

Table 1 shows the evaluation of the certification process regarding the ease of complying with the requirements of the standard, highlighting record management and training of employees as the most difficult aspects to comply. The other variables were easy to comply. Emphasis should be placed on the importance of observation and data collection to conduct productive and management analyzes based on real information that allows sound decision-making (Cerón-Muñoz *et al*., 2014).

The producers have the intention of getting recertified in GMP. The 97% of the owners considered that the satisfaction generated by farm changes achieved through GMP must be preserved and also that the recertification would allow them to abide by the norm while getting the benefits it offers.

The exploratory factor analysis produced three factors. Of these, two factors were chosen in which the 23 variables in analysis explained more than 65% of the factor. The variables "Satisfaction with the changes in the working conditions (S.TermsLabor)" and "Satisfaction with the changes in the relations with employees (S.Employees)" created the factor "Satisfied". The variables representing the ease of generating training programs for employees ("EaseTrainEmployee"), the ease of fulfilling the conditions required to be certified (EaseComplyTerms") and the ease of creating and implementing health programs (EaseHealth) contributed to the formation of factor "Ease".

Figure 1 represents the proposed structural equations. The "General satisfaction" is an exogenous variable because it was not altered by any of the variables in the dendogram. It can be noticed how satisfaction with GMP certification was explained by the presence of two constructs or latent variables (within circles): Satisfaction with changes ("Satisfied") and ease to obtain the GMP certificate ("Ease").

Table 1. Variables used to represent achievement, satisfaction with the changes, and ease of certification among GMP-certified milk producers in northern Antioquia.

Variable	Median	Average and deviation
Ease records	3	3.05 ± 0.97
Ease train employee	3	3.14 ± 1.40
S. employees	4	4.35 ± 0.89
Recognition	4	3.86 ± 0.89
Image	4	3.95 ± 1.18
Ease health	4	3.73 ± 0.99
Ease routine milking	4	4.16 ± 0.80
Ease comply terms	4	3.68 ± 1.29
Meet expectation	4	4.19 ± 0.78
S. program health	5	4.59 ± 0.50
S. milking	5	4.73 ± 0.65
S. management Waste	5	4.41 ± 0.69
S. cleanliness	5	4.73 ± 0.51
S. installations	5	4.54 ± 0.73
S. terms Labor	5	4.51 ± 0.80
S. wellbeing	5	4.65 ± 0.48
S. storage	5	4.51 ± 0.73
New knowledge	5	4.68 ± 0.67
New skills	5	4.41 ± 0.69
Happy goals	5	4.65 ± 0.72
Disposed change	5	4.62 ± 0.55
Pleased	5	4.49 ± 0.56
Competitive	5	4.51 ± 0.77

The "Satisfied" construct reflected two variables observed during data collection: the satisfaction of the producer with the changes in the working conditions of his employees (denoted as "S.TermsLabor"), and producer satisfaction with the changes in the relationship with employees after GMP certification (denoted as "S.Employees"). Importantly, both impact the construct with a factorial load higher than 0.70 and the error of these estimates is low (0.1 and 0.4, respectively). In other words, "satisfaction with changes in working conditions" and "satisfaction with changes in the relationships with employees" were the two aspects that satisfied GMP-certified producers.

The latent variable "Ease" is a reflection of three variables: The ability to train employees in the implementation of GMP ("EaseTrainEmployee"), the ease of fulfilling the conditions required by the GMP program ("EaseComplyTerms"), and the ease of creating and complying with the health programs required by the GMP model ("EaseHealth").

The ease to train workers in the implementation of GMP is the variable that most contributed to the creation of the construct "Ease", with a factorial load of 0.92. The ease to meet the requirements of the program has an acceptable factor load of 0.70, and the error is not high enough to reject its impact on the latent variable (0.5). The ease to create and comply with the health program has a low factor load (0.69) for the creation of the construct, implying that this is the least important of the three variables that make up this factor or construct.

Satisfaction with the adoption of GMP among milk producers in northern Antioquia is mostly explained by relationships with employees, such as: improvement of

working conditions, the ease of generating and accessing training programs for employees, and the satisfaction produced by the relationships established with them. For the producer, these three variables imply improvement of his image, fulfillment of the needs of acceptance, and fulfillment of the needs of self-realization (Maslow, 1943), since by guaranteeing his employees proper working conditions he improves his image before them and other producers. Improving relationships allows the producer to be more accepted by the stakeholders related to the farm and allows him to value his work.

The ease to comply with the requirements of the GMP program is a decisive factor when choosing to certify. A producer will feel satisfied of been certified if he feels it is easy to meet GMP conditions and to create and implement health programs. This is consistent with results by Radder and Bhanj (2011), who found that while a good payment per liter of milk produced under a clean production model is important, it is not the decisive factor for applying GMP.

According to our results, a producer is more likely to adopt GMP if the institution in charge of developing the program offers guidelines for the application and implementation of such models and the requirements are easy to adopt and apply. This is consistent with Nahuelhual *et al*. (2009) who state that application of GMP does not respond to economic motivations.

The adjustment statistics for the structural model were 0.96 CFI (which is within the ideal value; Bentler, 1990) and 0.094 RMSEA (less than 0.1, suggested as the maximum admissible value). Although this value is acceptable for evaluating the fit of the model, it should be taken into account that 37 surveys were applied and this parameter decreases as the sample space increases (Rigdon, 1996) (Figura 1).

Figure 1. Model of structural equations explaining satisfaction of milk producers certified in good management.

The recursive participation analysis allowed identifying that satisfaction with working conditions changes after certification and the ease of generating and accessing training programs for employees determine overall satisfaction. Satisfaction with the GMP certification program required that the producer scored his level of satisfaction with the changes in working conditions of his employees above 4.5 and valued the ease to train them above 2.5 (Figure 2).

Differences were found between municipalities when this variable was included in the analysis. Score less than 4.5 for "Satisfaction with changes in working conditions" was associated with Bello, Don Matías and Santa Rosa de Osos municipalities. Producers that showed greater satisfaction with the changes (greater than 4.5) -and thus greater general satisfaction- were associated with Belmira, Entrerríos, San José de la Montaña, San Pedro de los Milagros and Yarumal municipalities (Figure 3).

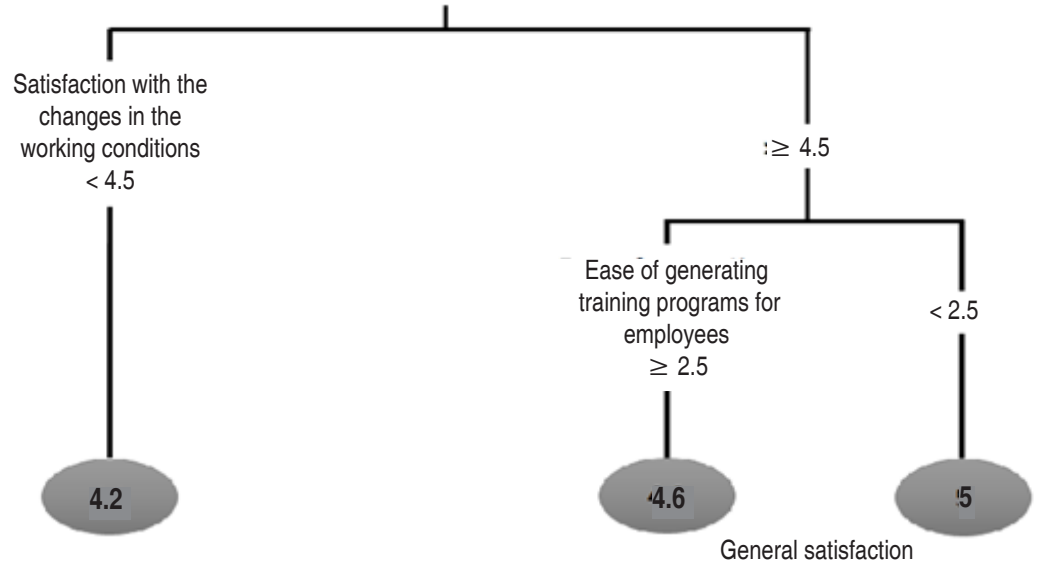

Figure 2. Criteria to determine general satisfaction in milk producers certified in good management practices in northern Antioquia..

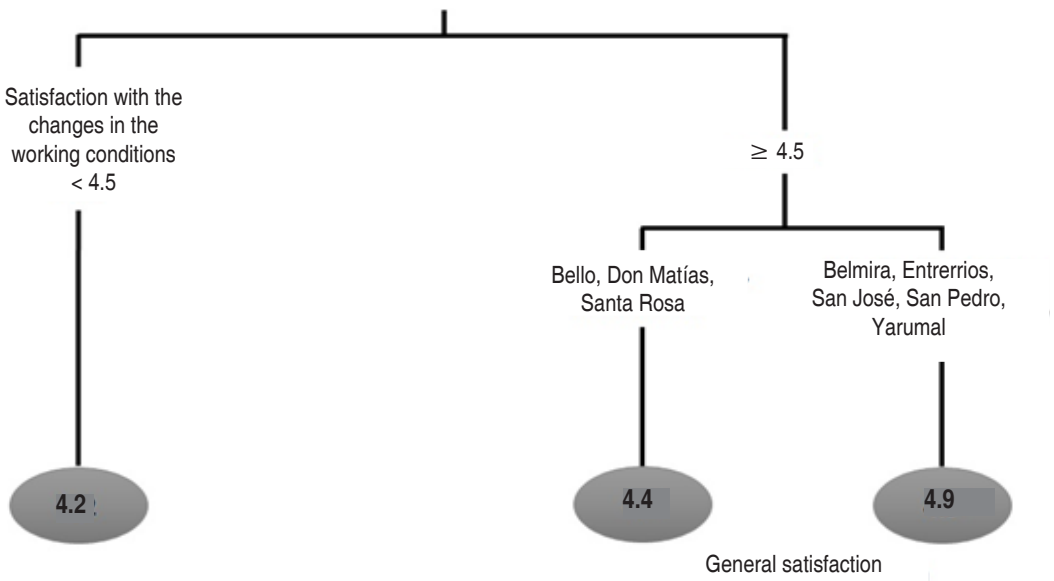

Figure 3. Overall satisfaction among milk producers certified in good management practices in northern Antioquia, discriminated by municipalities.

The CP (0.26) was used to prune the decision tree with the cross-validation method (Therneau *et al.*, 2015), resulting in a greater impact of variable "Satisfaction with changes in the working conditions of employees" on the overall satisfaction of producers (Figure 4).

The cross-validation CP of this model was 0.26, similar to the previous model. Thus, pruning was done on the first level of the tree, resulting –again- that satisfaction with changes in the working conditions of employees influenced more the general satisfaction of certified producers (Figure 4).

Some producers noted that potential bottlenecks to GMP certification are shortage of the ICA staff responsible for auditing the farms and differences in criteria between farm technical advisors and the ICA staff.

Figure 4. Satisfaction with changes in the working conditions of employees and its impact on the general satisfaction of milk producers certified in good management practices in northern Antioquia.

CONCLUSIONS

The adoption of GMP satisfies dairy farm owners, especially since it generates satisfaction with the resulting changes in the working conditions of employees, it facilitates permanent training, and their image improves before other producers and milk-buying companies.

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Pysicochemical properties and application of edible coatings in strawberry *Fragaria* × *Ananassa*) preservation

Propiedades fisicoquímicas y aplicación de recubrimientos comestibles en la conservación de fresa (*Fragaria* x *Ananassa*)

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Keywords:
Physico-chemical properties
Strawberries
Edible coating
Fruit conservation

ABSTRACT

The strawberry is one of the most economically important fruit in the Ecuadorian Highlands. Diseases are the main cause of post-harvest losses, causing damage to color, firmness and fruit quality. The main objective of this work was to evaluate the effect of three edible coatings based on gelatin, pectin and beeswax in the post-harvest conservation of the strawberry variety "Oso Grande" from the Chambo canton, Chimborazo province, Ecuador. The process was developed by using a completely random design with factorial arrangement: at room temperature and cooling First, the fruit was selected considering the degree of maturation, size, shape, health of the fruit, and uniform color. Next, it was separated into four groups, washed, and sanitized. Finally, the edible coatings based on gelatin, pectin and beeswax, all enriched with clove essential oil, were applied. The presented physical-chemical changes were evaluated in the two study temperatures. With the obtained results, it could be verified that the use of edible coatings affected statistically in the physical-chemical characteristics of the strawberry. The gelatin coating is the one with the best results, with a lower weight loss of 5.26%, firmness 9.92 N, soluble solids 7.49%, pH 3.69, acidity 0.73% and a shelf life of 5 days. The cost of production for obtaining the gelatin coating was the most economical with a price of $11.10/kg USD. The results show the efficiency of the edible coatings and the storage temperature in the extension of the shelf life of the strawberry.

Palabras clave:
Propiedades fisicoquímicas
Fresas
Recubrimientos comestibles
Conservación de frutas

RESUMEN

La fresa es una de las frutas económicamente más importante en la sierra ecuatoriana. Las enfermedades son las principales causas de pérdidas postcosecha, generando daños en el color, la firmeza y calidad del fruto. El principal objetivo de este trabajo corresponde a la evaluación del efecto de tres recubrimientos comestibles a base de gelatina, pectina y cera de abeja, en la conservación postcosecha de la fresa variedad Oso Grande proveniente del cantón Chambo provincia de Chimborazo-Ecuador. El proceso se llevó a cabo utilizando un diseño completamente al azar con arreglo factorial: a temperatura ambiente y de refrigeración. Los frutos fueron seleccionados en función del grado de maduración, tamaño, forma, sanidad de las frutas, y color uniforme; posteriormente se separó en cuatro grupos; se lavaron, desinfectaron y finalmente se aplicó el recubrimiento comestible a base de gelatina, pectina y cera de abeja, todos ellos enriquecidos con aceite esencial de clavo de olor. Se evaluaron los cambios físico-químicos presentados a las dos temperaturas de estudio. Con los resultados obtenidos se pudo comprobar que el empleo de recubrimientos comestibles afectó estadísticamente en las características físico-químicas de la fresa; siendo el recubrimiento de gelatina el que presenta los mejores resultados, con una menor pérdida de peso 5,26%, firmeza 9,92 N, sólidos solubles 7,49%, pH 3,69, acidez 0,73% y una vida de anaquel de 5 días. El costo de producción para la obtención del recubrimiento de gelatina fue el más económico con un precio de $11,10/kg USD. Los resultados obtenidos demuestran la eficacia de los recubrimientos comestibles y la temperatura de almacenamiento en la prolongación de la vida de anaquel de la fresa.

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FAO (2009) indicates that the post-harvest losses causes are very diverse. These include the collection at an inappropriate time in the ripening process. Also, the excessive exposure to rain, drought or extreme temperatures, contamination by microorganisms and physical damage contribute to reduce the product value (whose estimates are between 15% and up to 50% of production in developing countries). This is mainly because farmers do not have the knowledge and skills to apply good agricultural practices and much less about post-harvest manipulation.

The strawberry is a bright red fruit, succulent and fragrant that is obtained from the plant that receives the same name. Studies, like those of Barquero (2007), point out that the problem lies in the post-harvest losses (reaching up to 50% of production); and is usually caused by microorganisms. Since a strawberry is harvested in full maturation and maintained at room temperature, it deteriorates by 80%, in only 8 h (Wang and Gao, 2013). Works carried out by Bestfleisch *et al.* (2014), show that diseases are the main causes of post-harvest losses in the strawberry. The most frequent disease is the one caused by *Botrytis cinerea* (gray mold).

Pérez *et al*. (sf) say that new storage techniques have been developed in recent years, which make it possible to prolong the lifetime of fruits; one of these techniques is the use of edible coatings.

Edible coatings are defined as wrappers that cover the product, creating a semi-permeable barrier to gases (O_2 and CO_2) and water vapor; these improve the mechanical properties, helping to maintain the structural product integrity and to retain volatile compounds. One coating inside the fruit create a modified atmosphere that reduces the speed of respiration, and therefore delays the process of senescence (cell aging) (Pérez *et al*., sf). They also improve the mechanical properties, helping to maintain the structural product integrity that they cover, to retain volatile compounds and can contain food additives (antimicrobial agents, antioxidants, etc.) (Barquero, 2007; Anjum and Akhtar, 2012; Ruiz, 2015; Shao *et al*., 2015).

This research was carried out with the aim of improving the post-harvest management of the strawberry, using natural edible coatings such as gelatin, pectin and beeswax, combined with clove essential oil (the latter compound is very appreciated in the food industry because of its attributed antimicrobial and antifungal properties).

Former researches indicate that clove essential oil has proven very effective against *Penicillium digitatum* (Shao *et al*., 2015; Hall and Fernandez, 2004; Anjum and Akhtar, 2012). According Vesaltalab *et al*. (2012), similar results were obtained when used clove essential oil against *Botrytis cinerea*. Eugenol is the main chemical component of clove oil that has shown greater efficacy against several pathogens (Chaieb *et al*., 2007). Eugenol (4-allyl-2-methoxyphenol) presents antimicrobial properties, antioxidants, antifungal and antiviral activity. These can contribute to major alterations in the hyphal morphology, the alteration of the cell membrane and the release of cellular material from various fungi resulting in the death of these microorganisms (Shao *et al*., 2015).

The coatings used in the investigation are natural, do not cause harm to health, nor alter the sensory characteristics; this is beneficial for the consumer because the obtained product complies with all the quality standards for its commercialization (Campos *et al.,* 2011).

MATERIALS AND METHODS

Plant Material

The selected strawberry, during this work, was of the variety "Oso Grande", coming from the canton Chambo of the province of Chimborazo-Ecuador, whose location has a latitude of 1°44'00'' and a longitude of 78°35'00'. The selection was made according to Barquero (2007), considering the degree of maturation, size, uniformity and health of the fruit, without containing any dirt or foreign matter, in addition to having a minimum of 75% red coloration.

Preparation of the Coatings

The brands of the different substances used were: Gel'hada gelatin, Home Chef pectin and Apicare beeswax, the clove essential oil brand Isabrú Botanik with a 100% purity; distilled water with 98% of Purity Mark Prodont; Glycerin with a 99% purity Mark Ofmagnet and the CMC brand of magnets with a 99.5% purity. The formulation of edible coatings is shown in Table 1.

Table 1. Formulation of edible coatings.

Coatings	Comp.	Glic.	CMC	Pectin	AECO	A.D.
			(%)			
T0R (control)	0	0	0	0	0	0
T1R (gelatin)	3	0.75	0.75	0	1	94.50
T2R (pectin)	3	0.75	0.75	0	1	94.50
T3R (beeswax)	0.5	0.75	0	1.8	1	95.95
T0C (control)	0	0	0	0	0	0
T1C (gelatin)	3	0.75	0.75	0	1	94.50
T2C (pectin)	3	0.75	0.75	0	1	94.50
T3C (beeswax)	0.5	0.75	0	1.8	1	95.95

Room temperature R; Cooling temperature C; T0, T1, T2 y T3 Coatings used; Glic: Glycerin; CMC: Carboxymethylcellulose; AECO: Clove essential oil; A.D.: Distilled water.

The edible coatings were prepared by heating the distilled water at 65 - 75 °C, with constant stirring. The ingredients were added in the following order: The main component (gelatin, pectin or beeswax), glycerin, carboxymethylcellulose until a homogeneous mixture was obtained; finally, the temperature was reduced to 20 °C, and the clove essential oil was added. In the case of beeswax, it was first diluted at 62 - 65 °C, which was subsequently incorporated into the mixture with the other components.

The fruit was washed and disinfected by immersion in ozonated drinking water for 15 min. Then, left to dry; after that, the coating was applied by dipping the fruit and leaving it to drain for 1.5 min. Finally, it dried at temperature of 14 - 24 °C until its compaction. The storage was carried out on plastic trays, at an ambient temperature of 13 °C, with a relative humidity of 66.30%, and at refrigerated temperature of 4 °C, with a relative humidity of 95% (FRN – ESPOCH, 2016).

Physical and chemical properties assessment

Weight loss (%): performed by the gravimetric method, using a pioneer precision analytical balance. (Restrepo and Aristizabal, 2010).

Texture: the measurement of this property was done according to the INEN 1909 (2015); using a penetrometer mark QA. supplies, fruit pressure tester, FT 327 model and 3.5 mm diameter plunger.

Soluble solids (%): A refractometer: ATAGO brand, Model PAL1, was used as described in INEN 380 (1985).

pH: in this property measurement a potentiometer Testr 30 waterproof, Mark Oakton was used as described in the INEN 389 (1986). Titratable acidity (%) was performed according to the INEN 381 (1986), using an acid titrator: burette Dornic Marca BRIXCO (Camacho *et al*., 2009).

Lifetime Valuation

This was carried out by establishing Alvarado´s method, through the count of molds and yeasts (Alvarado, 1996).

Economic valuation

Production costs were determined based on projection for one year, according to the established FAO (2016a) method.

Experimental design

The experimental design was based on the evaluation of physical-chemical properties (VD: effects): weight loss (1); texture (2); soluble solids (%) (3); pH (4) and titratable acidity (% citric acid) (5) of the "Oso Grande" strawberry variety, in the post-harvest conservation through the addition of edible coatings, in front of a control. The process was carried out using a completely randomized design, with factorial arrangement, involving the following independent variables (VI): three types of edible coatings based on gelatin, pectin and beeswax; temperature: ambient and cooling; time: 11 days, with three replicates each. This same procedure was repeated, but with 12 levels for VI

Time. The results were subjected to analysis of variance and the means separation according to Tukey test.

The experimental treatments number for each VD measurement: physical and chemical properties: weight loss (1), texture (2), soluble solids (%) (3), pH (4) and titratable acidity (5) were calculated through the product independent variables levels (VI), according to the following mathematical model:

$$N_T = nT \,.\, nt \,.\, nTR \qquad (1)$$

Where:
N_T: Treatments number
nT: Levels number of the independent variable (VI): Time
nt: Levels number VI temperature: ambient (A) and refrigerated (R)
nTR: Levels number the VI type coating: gelatin, pectin and beeswax

Then, the total experimental runs number (NCET) was obtained by multiplying the number of treatments by the number of repetitions (coefficient k) (Equation 2).

$$N_{CET} = k \,.\, N_T \qquad (2)$$

By explicitly replacing expressions 1 and 2, the total treatments number and the experimental runs were obtained, respectively.

The total treatments number, according the modified Equation 1, in the measurement the physicochemical properties studied, is given according to the following mathematical models 3, 4 and 5.

$$N_{T\,VD\,1} = (nT.\, nt \,.\, nTR) \,.\, (TEx) = [(11) \,.\, (2) \,.\, (3) \,.\, (1)] = 66 \qquad (3)$$

$$N_{T\,VD\,2,3,4\text{ and }5} = (nT.\, nt \,.\, nTR) \,.\, (TEx) = [(12) \,.\, (2) \,.\, (3) \,.\, (4)] = [(72) \,.\, (4)] = 288 \qquad (4)$$

$$N_{TT\ \ VD\,1,2,3,4\text{ and }5} = N_{T\,in\,VD\,1} + N_{T\,VD\,2,3,4\text{ and }5} = 288 + 66 = 354 \qquad (5)$$

The experimental runs were performed with three replicates (coefficient k) for each VD. The total experimental runs value was given by the sum of the experiments, considering absence of control (Equation 6) and adding the total sum of control used; the value obtained is given after Equations 7 and 8.

$$N_{CT\text{ without }T\,VD\,1,2,3,4\text{ and }5} = (3) \,.\, (354) = 1062 \qquad (6)$$

$$N_{TM\,VD\,1,2,3,4\text{ and }5} = N_{CT\text{ without }T\,VD\,1,2,3,4\text{ and }5} + N_{T\ \ VD\,1,2,3,4\text{ and }5} \qquad (7)$$

$$N_{T\,samples\,VD:\,1,2,3,4\text{ and }5} = 1062 + 354^{*} = 1416 \qquad (8)$$

Where,
$N_{T\,VD1}$: Treatments measurement number the VD_1
$N_{T\ \ VD2,3,4\ \ and\ \ 5}$: Treatments measurement number the $VD_{2,3,4\text{ and }5}$
TEx: Type of Experiment, according to VD type
$N_{TT\,VD1,2,3,4\ and\ 5}$: Total Treatments number $VD_{1,2,3\text{ and }5}$
$N_{CTwithoutVD1,2,3,4\ \ and\ \ 5}$: Total runs number the $VD_{1,2,3,4\ \text{and}\ 5}$ measurement; no control treatment
$N_{Tsamples\ \ VD1,2,3,4\ \ and\ \ 5}$: Total Samples number used in all trials

RESULTS AND DISCUSSION

Assessment of physico - chemical characteristics

Weight loss (%). In the analysis of the weight loss variable, referential studies, such as those performed by Robinson *et al*. (1975) report that the maximum percentage of weight loss, for the marketing of the strawberry is 6%. In this research, these values are within the established parameter up to the third and fourth day (which correspond to the treatments in beeswax and gelatin refrigeration respectively). Other treatments are outside the set parameter (from the third day). Weight loss values (from day 2 to day 12) are shown in Figure 1. The results were re-obtained from the second day, since the initial measurements (first day) constitute the starting point for this research.

The treatment with gelatin was the one that presented the best characteristics in this parameter, since its weight loss percentage only reached a value of 5.26. This is because gelatin is a hydrocolloid substance (gelling agent), which gives the coating thickening

properties that do not allow the fruit to lose the water it has in its composition because it forms a good barrier to the transfer of gases and water vapor (Trejo *et al*., 2007).

Figure 1 shows a considerable weight loss of the treatments at room temperature in relation to those of refrigeration. This is because, after fruits are harvested, physiological changes occur, such as perspiration (Fernández *et al.,* 2015); and because the fruit is constituted mainly of water (72 - 95%). There is a great dehydration or wilting, which controlled by refrigeration avoids the loss of water that is produced by evaporation (Pelayo and Castillo, 2002).

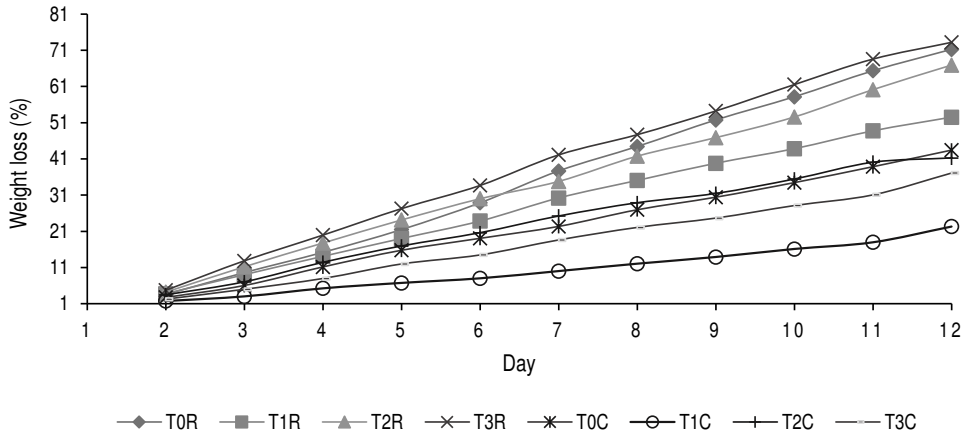

Figure 1. Weight loss depending on the experimentation days at room temperature and refrigeration.

Texture. The loss of firmness constitutes one of the most significant physiochemical changes; it is associated with the loss of water, generated through perspiration and respiration of the fruit. As a result, wilting and loss of consistency occur. It is one of the main factors used to determine fruit quality and post-harvest shelf life (Acuña, 2009).

With regard to the variable texture, there are investigations, such as Alcántara (2009), where they indicate that, for its commercialization, the strawberry must have a firmness oscillating between 9.8 – 11.5 N. In the present investigation, at refrigeration temperature, it is within the parameter until the fifth day in the treatments coated with beeswax, pectin and control and until the sixth day with the treatment of gelatin.

Gelatin coatings are those that delayed the softening of the fruit longer, presenting a value (per day six) of 9.92 N. This was because the gelatin develops excellent mechanical properties that are very favorable to cover food (Acosta, 2014); in this order, treatments with pectin and beeswax were followed which (until day five) presented a value of 10.02 N; because polysaccharides, such as pectin, are capable of constituting a structural matrix, allowing to obtain edible coatings with moderate mechanical properties (Eum *et al*., 2009). In the case of beeswax, these results were due to the addition of polysaccharide (CMC) in the formulation of the treatment, which provided good mechanical properties since lipids, on their own, present drawbacks in this aspect (Campos *et al*., 2011; Pavón and Valencia, 2016).

Analyzing temperature factor, it can be observed that the effect of refrigeration presented better results with respect to the ambient temperature treatments, obtaining values similar to those reported by Trejo *et al*. (2007) in his research. For this reason, the application of 1% of gelatin for edible coatings in strawberries influenced the softening of tissues; since the uncoated fruits showed a 62% loss of firmness; meanwhile, the coated strawberries showed 5% values from the third day of storage. In Figure 2, it can be seen that the texture decreases with storage time, until finally softening is achieved.

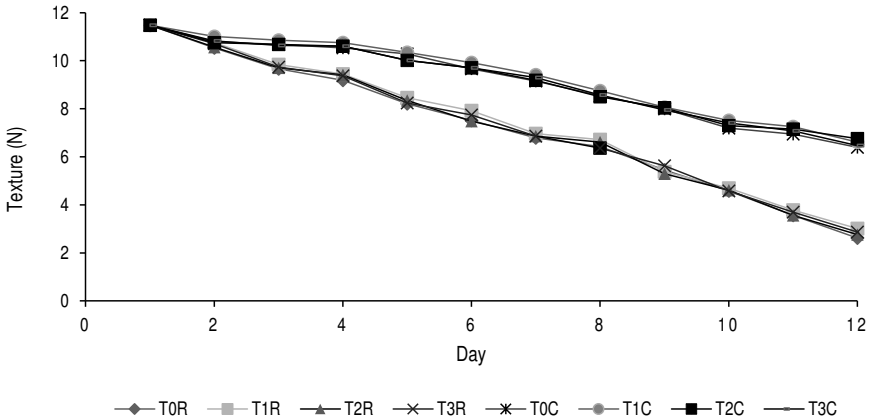

Figure 2. Texture Behavior (N) as a function of elapsed time at room temperature and refrigeration.

Soluble solids (%). For the soluble solids variable, researches such as that of Chicaiza (2015), assert that for the "Oso Grande" variety, this parameter must have a minimum value of 6.91 ° Bx; while Reyes and Zschau (2012) confer a maximum value of 8.2 °Bx, so that the fruit is acceptable. In this research, these values are within the established data until the twelfth day (in the treatments in gelatin refrigeration, beeswax and without coating), and the pectin until the eleventh day. The percentages of soluble solids obtained in the research are shown in Figure 3.

The treatments with gelatin kept stable the amount of soluble solids present in the strawberry, reporting a percentage of 7.49 because the gelatin, when presenting excellent barriers to oxygen, slows down the process of degradation of sucrose that cause fungi, thus avoiding an increase in soluble solids. Refrigeration (considering the temperature factor) maintains the activity of the microorganisms in the latent state, which allows a lesser variation of soluble solids present in the fruit (Ruiz, 2015). The soluble solids variation can be seen in Figure 3.

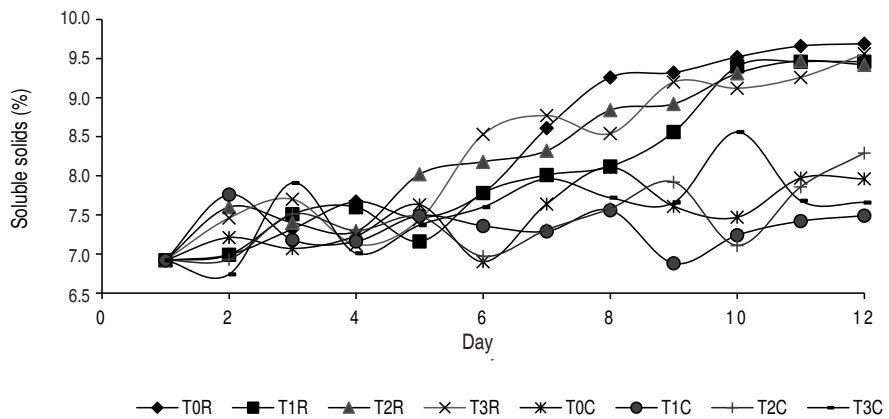

Figure 3. Soluble solids (%) depending on the experimentation days at room temperature and refrigeration.

pH and titrable acidity. In regard to the pH variable, reference studies such as those of Nunes (2007) report values between 3.4 – 3.75. In this research these values remained until the eighth day, which correspond to the treatments that are at room temperature, but until day 12 for those that are in refrigerated conditions (this was because

bacteria and fungi use, as a source of energy and nutrients, the components present in food, such as sugars, amino acids, and phenolic compounds). In this case, organic acids or glucose may be produced. The production of these compounds causes an increase in the concentration of OH-ions, which results in an increase in pH. This type of process is what causes food to deteriorate (Fennema, 1996).

The gelatin-based coatings maintain the pH of the strawberry stable, reporting a value of 3.69, since the gelatin forms a protective layer, avoiding the entry of oxygen and stopping the activity of the fungi and bacteria, which keeps the pH in the fruit stable. In Figure 4 it is possible to observe the increase of the pH of the treatments at room temperature with respect to those of refrigeration.

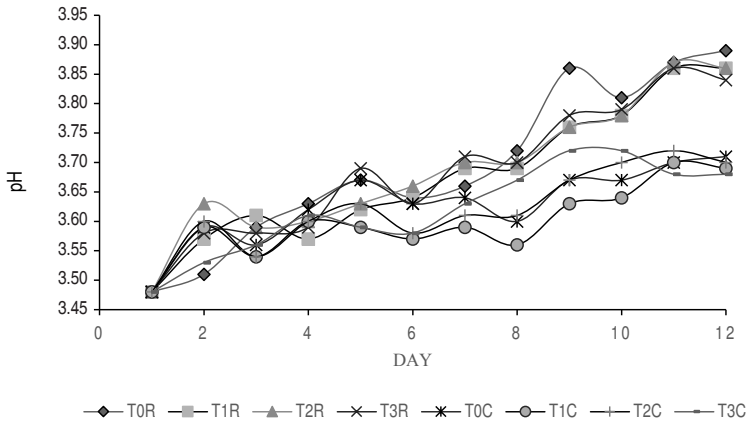

Figure 4. pH behavior as a function of elapsed time at room temperature and refrigeration.

As for the titratable acidity variable, researches such as that of Chicaiza (2015), indicate that the strawberry should have a minimum percentage of 0.69 g and a maximum of 0.89 g of citric acid per 100 g of product. In this research these values are within the established data until the ninth day; which corresponds to the treatments in gelatin refrigeration, pectin, and beeswax with percentages of 0.73, 0.71 and 0.69, respectively. This was due to the conservation effect provided by the low temperatures; the coatings create a protective layer on the fruit that retains volatile compounds and slows its senescence process, stabilizing the organic acids longer (ELIKA, 2010; Villegas and Albarracín, 2016).

The acidity reduction coincided with the pH increase. The cause was the use of organic acids as an energy source to support the process of senescence of the fruit (Chicaiza, 2015). In addition, it must be considered that the organic acids present in the food influence the taste, color and stability of the same. Acidity values can be very variable, as in fruit. Citric acid can constitute up to 60% of the total soluble solids in the edible portion (Figure 5).

Molds and yeasts. In regard to the parameter molds and yeasts, reference studies such as those carried out by Frazier and Westhoff (2003); report that in order for a fresh fruit to be consumed, without causing any health risk, it must possess a range acceptable to oscillate between 3 log CFU mL^{-1} and 4 log CFU mL^{-1}. In this research, these values remain until the ninth day in treatments with refrigerated coatings; other treatments are outside the parameter set from the third day.

The incorporation of lipids, such as essential oils in an edible coating, is an effective method to solve some of these problems, as well as in the control of fungal diseases of the fruit (Pontigo-Suárez *et al.,* 2015) by reducing the diffusion processes and the maintenance of high concentrations of active molecules on the surface of the fruit (Shao *et al.*, 2015). Clove essential oil has been shown to have antimicrobial, antifungal, and antiviral activity due to its main chemical component, which is eugenol (Hall and Fernandez, 2004; Anjum and Akhtar, 2012; Vesaltalab *et al*, 2012; Chaieb *et al.*, 2007; Shao *et al.*, 2015).

The gelatin coatings presented the lowest microbial load, with a value of 45 CFU mL^{-1} because when combined with a protein, a polysaccharide and a lipid, this helps to minimize the disadvantages of the individual components,

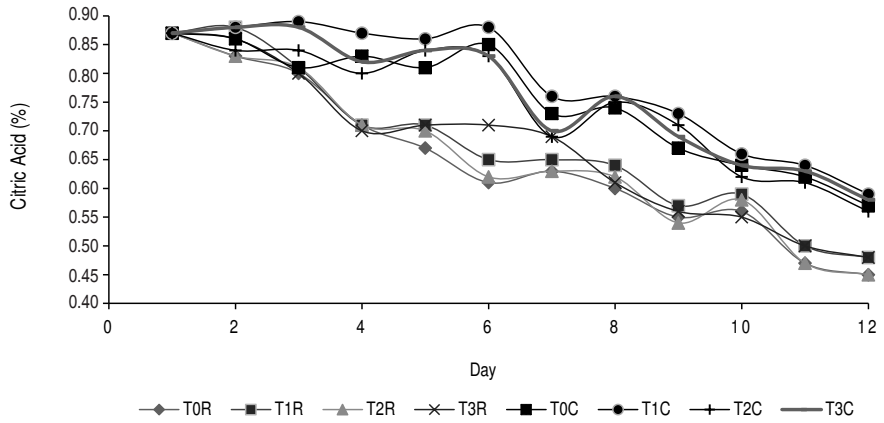

Figure 5. Citric acid (%) as a function of elapsed time at room temperature and refrigeration.

making synergy of their functional and physical properties (Pascall and Lin, 2013).

Beeswax, as a coating, presented good characteristics within this parameter, with a value of 51 CFU mL^{-1} because, when using lipids such as beeswax and essential oils, combined with polysaccharides such as CMC, coatings are obtained with excellent barriers against oxygen, carbon dioxide and moisture (Parzanese, 2009).

Treatments with pectin followed, in numerical value, to beeswax with a value of 52 CFU mL^{-1}; since, when using polysaccharides in combination with essential oils, a good combination is produced, obtaining edible coatings with good barriers against humidity and moderate mechanical properties (Parzanese, 2009; Ruiz *et al.,* 2016).

The conservation in low temperatures is essential to avoid the development of molds and yeasts, especially the development of the mold *Botrytis cinerea*, since according to Shao *et al.* (2015); it is this mold that most affects the strawberry (grows at temperatures of 15 - 20 °C and in conditions of high relative humidity with values between 85 – 90%). The temperature directly affects the conservation of the food, it already keeps the microorganisms in latent life (inactivity), thus increasing the useful life of the product (ISETA, sf.). This is demonstrated in the results of this research, since refrigeration achieved up to 50% fungal growth, compared with treatments at room temperature (Figure 6).

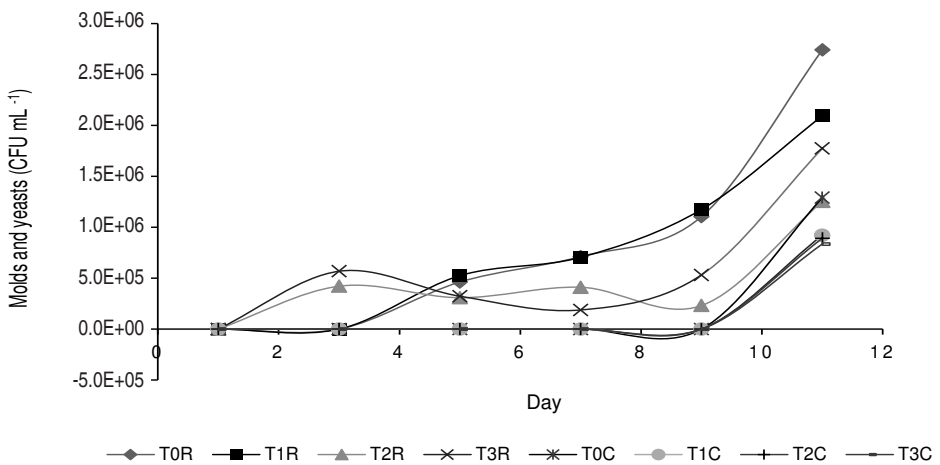

Figure 6. Growth of molds and yeasts as a function of elapsed time at room temperature and refrigeration.

Value of shelf life

Edible coatings at room temperature reported values between 2 to 3 days of shelf life. These data are consistent with those of the FAO (2016b); which states that the strawberry is very perishable and deteriorates within 2 or 3 days of harvesting in natural environmental conditions.

In the treatment of refrigerate fruit, values of 4 to 5 days were obtained. These values exceed that indicated by Acuña and Llerena (2001); which shows that the strawberry lasts up to four days in refrigeration; this verifies the effectiveness of the use of coatings in the post-harvest handling of this valuable fruit (Villegas and Albarracín, 2016)..

The useful life of the strawberry can improve by performing a good post-harvest management, avoiding in this way the activation of metabolic processes that cause the deterioration of the fruit. Temperature is a very important factor for storage conservation. At high temperatures, senescence processes develop at high speed, and at low temperatures, metabolic activity and moisture loss is reduced, slowing microbial proliferation. For this reason, the preparation of the fruit at refrigerated temperatures after collection is recommended (Ariel, 2004). The values used for the calculation of useful life can be seen in Table 2.

Economic valuation

All production costs were projected for one year, with a production capacity of 300 kg of strawberry daily,

Table 2. Values used for the calculation of useful life of the strawberry.

	Molds and yeasts							
	Room temperature				Cooling temperature			
Time (days)	T0R	T1R	T2R	T3R	T0C	T1C	T2C	T3C
1	3.19	3.19	3.19	3.19	3.19	3.19	3.19	3.19
3	3.50	3.22	12.70	12.60	3.09	3.04	3.18	3.30
5	12.96	13.25	13.28	12.76	3.66	3.76	3.53	3.04
7	13.16	13.78	14.27	14.28	3.74	3.47	3.22	3.30
9	14.33	13.30	13.78	13.33	12.02	3.81	3.95	3.93
11	15.12	14.79	14.60	14.85	14.36	14.07	13.93	14.08
Shelf life (days)	3	3	2	2	4	5	5	5

Table 3. Production costs for one year (USD).

	Covering		
Concept	Gelatin	Pectin	Beeswax
Direct materials	174,144.43	174,171.32	174,163.41
Direct labor	12,413.26	124,13.26	12,413.26
Indirect costs of production	29,280.46	29,280.46	29,280.46
TOTAL $USD	**215,838.15**	**215,865.04**	**215,857.13**

using 75% capacity. In Table 3, production costs can be seen for obtaining the three types of coatings.

CONCLUSIONS

In this research the physicochemical parameters of weight loss, texture, soluble solids, pH and acidity proved the efficacy of edible coatings in the post-harvest management of the strawberry variety “Oso Grande”. The use of edible membranes, with bioactive agents such as cloves and low temperatures, allowed extending up to a maximum of five days the physicochemical and sanitary characteristics of the fruit under study.

Based on the results obtained in the physicochemical and microbiological analyses, the following results were determined: the gelatin coating presents the best characteristics with a lower percentage of weight loss (5.26), a delay in the softening of the texture (9.92 N), slowing of senescence maintaining the stability of soluble solids (7.49%), pH (3.69), acidity (0.73%) and better inhibition of the proliferation of molds and yeasts into the strawberry.

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ÍNDICE DE AUTORES

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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 debe remitir el formato "Autorización para Publicación de Obras y Cesión de Derechos Patrimoniales", en el 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. Las formas de publicación son: artículos de investigación científica y tecnológica, artículos de revisión, artículos de reflexión 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 Agrícolas, Producción Animal, Forestales y de Ingeniería Agrícola y de Alimentos. La extensión no debe exceder de 5.200 palabras, las hojas deben ser tamaño carta, escritas a interlineado sencillo, 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 cuando se requiera 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. Cuando se requiera se debe 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 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 mínimo cuatro, 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

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

Se deben describir en forma clara, concisa y secuencial, los materiales 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. 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.

Literatura citada

Sólo se listan las referencias bibliográficas mencionadas en el texto. No se aceptan notas de clase, artículos en preparación o en prensa, o cualquier otra publicación de circulación limitada. Se debe evitar el exceso de autocitas.

La bibliografía se deberá incluir al final del texto, sólo con las referencias citadas en el mismo. Se debe incluir el número doi asignado a cada artículo consultado. Las citas en el texto deben incluir apellido del autor y año, con coma entre autor y año. Ejemplo: Pérez, 1995; además conservar el siguiente orden de citación:

- 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 y. Ejemplo: Gil y 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.* que significa y otros; en la bibliografía se deben citar todos los autores.

Las comunicaciones personales, se deben citar al pie de la página y no se incluyen en la bibliografía.

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, de 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 la publicación original.

En cada referencia para todos los autores cite primero el apellido seguido de la inicial del nombre sin puntos, separando autores con coma y espacio.

Ejemplos:

Para libros: Autor (es). Año. Título del libro, edición, ciudad de su sede, casa editora y, 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.

Para capítulos de libros: Autor (es). Año. Título del capítulo, páginas consultadas (pp. # - #). En: Apellidos y nombres de los compiladores o editores (eds.), título del libro, edición, casa editora y 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.

Para artículos de revistas: Autor (es). Año. Título del artículo, nombre completo de la revista volumen(número): 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. Santa Fé de Bogotá.

Tesis, trabajos de grado. Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae). Tesis Magister en Bosques y Conservación Ambiental. Facultad de Ciencias Agropecuarias. Universidad Nacional de Colombia. Medellín. 78 p.

Abril G. 2002. Biogeografía y descripción de las especies del género *Collaria* sp. en seis zonas lecheras del Departamento de Antioquia. Trabajo de grado Ingeniería Agronómica. Facultad de Ciencias Agropecuarias. Universidad Nacional de Colombia. Medellín. 49 p.

Cita de cita. Magalhaes LM e da Cruz AJ. 1979. Fenologia do pau-rosa (*Aniba duckei* Kostermans), Lauraceae, em floresta primária na Amazônia Central. Acta Amazónica 9(2): 227- 232. Citado por: Gómez CP. 2004. Autoecología del mortiño (*Vaccinium meriodinale* Swartz Ericaceae). Tesis Magister en Bosques y Conservación Ambiental. Facultad de Ciencias Agrarias, Universidad Nacional de Colombia. Medellín. 46 p.

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 Supl. 2: 195-201.

Para 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. En: Agricultura Tropical, http://agrotropical.edunet.es. 25 p.; consulta: noviembre 2003.

PUBLISHING POLICY

REVISTA FACULTAD NACIONAL DE AGRONOMÍA MEDELLÍN

The National Faculty of Agronomy Journal (RFNA) is published by the Faculty of Agricultural Sciences of Universidad Nacional de Colombia - Medellín. It is aimed at teachers, 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 is a four-monthly publication at national and international level. Its aim is to disclose original and unpublished 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.

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.

Critical reflection articles: A document presenting completed research results from an analytical, interpretive or critical author's point of view, on the specific issues already mentioned, using original sources.

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 Medellín 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.

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INSTRUCTIONS TO AUTHORS

General guidelines

Papers can be sent by email to: rfnagron_med@unal.edu.co, or 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. Letter of originality which accepts no simultaneous nomination of the article to other journals or publishers and assigns and the "Autorization for Release of Works and Economic Rights Assignment" should be attached. 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, it must be letter-size sheets, typed double-spaced, 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 2002a2005, 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, Spanish or Portuguese. 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 researches, 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.

Cited Literature

Only bibliographical references cited in the text are listed. Lecture notes, articles in preparation or in press, or any other publication with limited circulation are not accepted. Excessive self-citation should be avoided.

The bibliography should be included at the end of the text, containing only the references cited in it, including the doi number. Citations in the text should include author's surname and year, with comma between author and year. Example: Pérez, 1995. They should also keep the following citation order:

- 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 by 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 and replace the others by the Latin expression *et al.,* which means and others. All authors should be mentioned in the bibliography.

Personal communications should be cited at the bottom of the page and not included in the bibliography.

Bibliographic references are ordered alphabetically by first author's surname, without numbering and without indentation. To cite several publications by the same author, chronological increasing order must be followed. Alphabetical order of titles must be followed in case they are from the same year.

The references should be arranged alphabetically by first author, without numbers and without indentation. To cite several publications by the same author, follow the chronological up order, if they are of the same year, follow the alphabetical order of the titles.

References should contain all the data allowing to its easy location.

Examples:

For books: Author (s), year. Book title, edition, place of publication, publisher and pages consulted (pp. # - #) or total pages (# p.). 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.

For book chapters: Author (s), year. Chapter title, pages consulted (pp. # - #). En: Surnames and names of the editors or publishers (eds.), book title, edition, publisher and place of publication, total pages (# p.). Example: Bernal H. 1996. Chapter 6: Evapotranspiración. pp. 112-125. In: Agrios G. (ed.). Fitopatología. Second Edition. Editorial Limusa, México D.F. 400 p.

For journals: Author (s), year. Article title, journal full name volume(number): page-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. Geotechnical engineering and environmental protection. p. 65-94. In: Memorias IX Colombian Congress of Soil Science. Colombian Society of Soil Science. Santa Fé de Bogotá.

Theses and dissertations: Gómez C. 2004. Autoecología de mortiño (*Vaccinium meridionale* Swartz Ericaceae). Master's Thesis in Forestry and Environmental Conservation. Faculty of Agricultural Sciences. Universidad Nacional de Colombia. Medellín. 78 p.

Abril G. 2002. Biogeografía y descripción de las especies del género *Collaria* sp. en seis zonas lecheras del departamento de Antioquia, Dissertation. Faculty of Agricultural Sciences. Universidad Nacional de Colombia. Medellín. 49 p.

Citation of a citation: Magalhaes LM e da Cruz AJ. 1979. Phenology do pau-rosa (*Aniba duckei* Kostermans), Lauraceae, em floresta primária na Amazônia Central. Acta Amazónica 9(2): 227-232. Cited by: Gomez CP. 2004. Autoecología de mortiño (*Vaccinium meriodinale* Swartz Ericaceae). Master's Thesis in Forestry and Environmental Conservation. Faculty of Agricultural Sciences, Universidad Nacional de Colombia. Medellín. 46 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. In: Tropical Agriculture, http://agrotropical. edunet.es. 25 p.; accessed: November 2003.

POLÍTICA EDITORIAL

REVISTA FACULTAD NACIONAL DE AGRONOMÍA

A Revista Facultad Nacional de Agronomía é uma publicação da Facultad de Ciencias Agrarias da Universidad Nacional de Colombia – Sede Medellín. Orienta-se a professores, pesquisadores, estudantes e a todos os profissionais que criam conhecimento e articulam a ciência e a tecnologia para fazer o campo mais produtivo no âmbito empresarial e da economia camponesa.

A periodicidade da Revista é cuatrimestral, com circulação nacional e internacional e seu objetivo é divulgar artigos originais e inéditos de caráter científico que respondam perguntas específicas e forneçam suporte e provas a uma hipótese, em aspectos relacionados com das Ciências Agronômicas, Zootecnia, Ciências Florestais e Engenharia Agrícola e de Alimentos e disciplinas afins que contribuam à solução dos limitantes do agro no trópico.

Levando em conta os critérios considerados por Colciencias, a revista considera documentos das seguintes tipologias:

Artigos de pesquisa científica e tecnológica: Documentos que apresentam, de forma detalhada, os resultados originais de projetos de pesquisa concluídos. A estrutura utilizada contém, geralmente, quatro partes fundamentais: introdução, metodologia (materiais e métodos), resultados e discussão, e conclusões.

Artigos de revisão: Documentos produto de uma pesquisa concluída onde são analisados, sistematizados e integrados os resultados de pesquisas publicadas ou não publicadas, sobre um campo em ciência e tecnologia, a fim de dar conta dos avanços e tendências de desenvolvimento. Caracteriza-se por apresentar uma cuidadosa revisão bibliográfica de pelo menos 50 referências.

Artigos de reflexão: Documentos que apresentam resultados de pesquisa concluída com uma perspectiva analítica, interpretativa ou crítica do autor, sobre os temas específicos antes mencionados, recorrendo a fontes originais.

Artigos curtos: Documentos breves que apresentam resultados originais preliminares ou parciais de uma pesquisa cientifica ou tecnológica, e que geralmente precisam de uma rápida difusão. Para todos os casos o 60% das citações deve provir de artigos publicados nos últimos dez anos.

Os artigos devem ser apresentados de acordo com os parâmetros estabelecidos nas "Instruções para os Autores", aqueles que não seguirem as normas básicas não serão considerados para publicação. Deve preencher o formulário "Autorização para Publicação de Obras e Sessão de direitos" a qual será fornecida pela Revista. O formulário é explícito enquanto que todos os autores estão informados do envio do artigo para a Revista, além de estar de acordo com ele. Também o formulário indica que não se apresentam conflitos de interesse entre eles e expressam que o conteúdo do manuscrito não tem sido nem será enviado para a sua publicação em outra revista.

O Comitê Editorial, junto a uma equipe de editores asociados, avaliará o mérito científico do documento e o submeterá a avaliação na modalidade duplo-cego–isto é, tem- se estrito anonimato da relatoría- por dois árbitros especializados no assunto, um deles nacional e outro internacional, os quais entregarão seu ditame no formato estabelecido pela Revista.

O Comitê Editorial reserva-se o direito de aceitar ou não as colaborações. O ditame após o processo de revisão pode ser: aceito para publicação com nenhuma ou poucas modificações; aceito para publicação com modificações maiores segundo as observações dos avaliadores; reconsiderado para publicação se modificado substancialmente –neste caso será catalogado como material novo-; recusado para publicação. Caso o artigo for aceito, este será devolvido aos autores para correção e remitido novamente ao Diretor da Revista nos seguintes 30 dias calendário.

A impressão de gráficos, figuras ou fotografías em cor é opcional e tem custo adicional por página precisava de cem mil pesos colombianos (100 mil pesos). A redação da Revista reserva-se o direito de realizar modificações de forma no texto do artigo (títulos, resumos/abstracts, tabelas e figuras), embora, sempre que seja possivel, os autores serão consultados a respeito das mudanças introducidas.

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A Revista admite comentários e opiniões contrárias aos termos emitidos no material publicado, aceita retratações argumentadas dos autores e corrigirá os erros tipográficos e de outra índole que surgirem na publicação de um artigo. A Facultad de Ciencias Agrarias e a Revista não se responsabilizam ou solidarizam, necessariamente, com os conceitos emitidos nos artigos publicados, cuja responsabilidade será totalmente do autor ou autores.

Para maior informação, correspondência, assinaturas e troca, endereçar-se a 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; correio eletrônico: rfnagron_med@ unal.edu.co A versão eletrônica da Revista pode ser consultada em http://www.revistas.unal.edu.co/index.php/refame

INSTRUÇÕES PARA OS AUTORES

Parâmetros gerais

Os artigos podem ser enviados ao endereço eletrônico: rfnagron_med@unal.edu.co ou também ingressando no site das Revistas da Universidad Nacional de Colombia usando o programa Open Journal System http://www.revistas.unal.edu.co/. Serão considerados apenas os artigos escritos em Inglés. Junto com o trabalho deverá encaminhar o formulário "Autorização para Publicação de Obras e Sessão de direitos" no qual se aceita a não postulação simultânea do artigo a outras revistas ou órgãos editoriais e cedem-se à Revista os direitos de difusão. As formas de publicação são: artigos de pesquisa científica e tecnológica, artigos de revisão, artigos de reflexão e artigos curtos. Os artigos podem ser elaborados por professores e/ou pesquisadores da Universidad Nacional de Colombia, ou qualquer outra instituição afim, nacional ou internacional, nos temas agropecuários, florestais, e de engenharia agrícola e de alimentos. A extensão não deve superar as 5.200 palavras, as folhas devem ser tamanho carta, escritas a duplo espaço, letra ou fonte Times New Roman ou Verdana, tamanho 12 pontos, margens de 3 cm na parte superior, 2 cm na inferior e 2,5 cm nas margens laterais direita e esquerda. As tabelas e figuras (isto é, gráficos, desenhos, esquemas, diagramas de fluxo, fotos e mapas) devem aparecer em folhas independentes e com numeração consecutiva (Tabela 1... Tabela n; Figura 1... Figura n. etc.). Os textos e tabelas devem ser apresentados no processador de palavras MS-Word®; as tabelas e diagramas de freqüência (gráficos de barras e de pizzas) originais devem aparecer tanto no arquivo do manuscrito quanto no original de MS-Excel®; outras figuras, como fotos sobre papel e desenhos, podem ser enviadas em original ou digitalizadas, e remitidas no formato digital de compressão JPG (ou JPEG) preferivelmente com uma resolução de 600 x 600 dpi (mínimo 300 dpi); é desejável que as fotos originais sejam enviadas como slides. Como norma geral, só serão aceitas tabelas e figuras em preto e branco; imagens coloridas serão incluídas só em caso estritamente necessário e a juízo do Comitê Editorial.

Unidades, abreviaturas e estilo

Deve utilizar-se o Sistema Internacional de Unidades (SIU), e aquelas unidades específicas de maior uso por parte da comunidade científica. Quando seja necesario debe-se usar a forma exponencial Exemplo: kg ha^{-1}. O significado das abreviaturas deve ser citado por extenso quando mencionadas por primeira vez no manuscrito. O estilo da escrita deve ser absolutamente impessoal, em tempo gramatical pretérito na introdução, procedimentos e resultados, e presente na discussão, evitando a conjugação de verbos em primeira ou terceira pessoa do singular ou do plural.

Os números de um a nove devem-se escrever em palavras, exceto quando refletem ou indicam unidades de medida ou se colocam vários números consecutivamente Exemplo: "oito tratamentos", "3, 7 y 9 leituras", "15 kg". Deve-se utilizar o zero antes do ponto decimal. Para separar intervalos de um o mais anos, deve-se usar a letra "a", e hífen para períodos de crescimento (safras). Exemplo. Período 2002 a 2005, safras 1999-2000, 2000-2001.

Título e autores

O título do artigo não deve incluir abreviaturas e é obrigatória sua tradução ao inglês. Sempre que possível, o título não deve superar as 15 palavras e deve refletir com precisão o conteúdo do documento. Em caso de conter nomes científicos de espécies vegetais ou animais, estes devem ir em itálica minúscula, com maiúscula somente a primeira letra do gênero e do classificador. Embaixo do título em inglês escreve-se o nome(s) e sobrenome(s) dos autores, sem seus títulos acadêmicos, nem cargos laborais, numa linha horizontal e conforme a sua contribuição à pesquisa e/ou preparação do artigo.

Na parte inferior da primeira página, como nota ao rodapé, escreve-se o cargo laboral dos autores, o nome e a cidade onde se localiza a entidade para a qual trabalham ou do patrocinador para a realização do trabalho e o correspondente endereço eletrônico. Adicionalmente, deve anexar-se um resumo do currículo dos autores, onde se mencionem os artigos publicados em outras revistas.

Resumo, abstract e palavras-chave

O resumo não deve superar as 250 palavras escritas num único parágrafo. Deve ser redigido em espanhol, inglês ou português. Deve conter em forma breve justificação, objetivos, métodos utilizados, resultados obtidos mais relevantes e conclusões. É obrigatório acompanhar o resumo com um máximo de seis palavras-chave, traduzidas ao inglês (key words), diferentes às utilizadas no título. Aceitam-se como palavras-chave não somente palavras simples, mas também termos compostos por até três palavras. Estas devem ir escritas em minúscula e separadas por vírgulas.

Introdução

O título não é obrigatório. Define o problema e informa sobre o estado da arte a respeito do tema principal do artigo, além disso, indica as razões que justificam a pesquisa e propõe os objetivos da mesma. É obrigatório acompanhar os nomes vulgares com o nome(s) científico(s) e a abreviatura(s) do classificador na primeira menção dentro do texto. Não mencionar marcas de produtos, mas nomes genéricos ou químicos.

Materiais e métodos

Aqui devem ser descritos em forma clara, concisa e seqüencial, os materiais (vegetais, animais, implementos agrícolas ou de laboratório) utilizados no desenvolvimento do trabalho, assim mesmo mencionam-se os aspectos relacionados com a localização, preparação e execução dos experimentos. Devem indicar-se o desenho escolhido, as variáveis registradas, as transformações feitas aos dados, os modelos estatísticos usados e o nível de significância empregado. Evitar detalhar procedimentos previamente publicados.

Resultados

São a parte central do artigo, devem ir respaldados por métodos e análises estatísticas apropriadas. Devem apresentar-se de maneira lógica, objetiva e seqüencial mediante textos, tabelas e figuras; estes dois últimos apoios devem ser de fácil leitura, interpretáveis de forma autônoma e ir citados sempre no texto. As tabelas devem conter poucas colunas e linhas. É preciso incluir o nível de significância estatística representado por letras minúsculas do começo do alfabeto (a, b, c, d,...), asterisco simples (*) para $P< 0,05$, duplo asterisco (**) para $P< 0,01$ ou três asteriscos (***) para $P<0,001$. As pesquisas que não obedecem um

desenho estatístico devem mostrar a informação de forma descritiva. Deve-se utilizar subíndice para modificações, os superíndices devem ser utilizados para potencias ou notas ao rodapé em tabelas e figuras.

Discussão

Refere-se à análise e interpretação objetiva dos resultados, confrontando-os com os resultados obtidos em outras pesquisas, ou com os fatos ou teorias conhecidas sobre o tema. Explica os resultados, particularmente quando diferem da hipótese proposta. Destaca a aplicação prática ou teórica dos resultados obtidos e as limitações encontradas. Ressalta a contribuição a uma determinada área do conhecimento e o aporte à solução do problema que justifica a pesquisa. Finalmente, proporciona elementos que permitem propor recomendações ou lançar novas hipóteses. Não devem ser feitas afirmações que vão além do que os resultados podem apoiar.

Conclusões

São as afirmações originadas a partir dos resultados obtidos, devem ser coerentes com os objetivos propostos e a metodologia empregada; adicionalmente, expressar a contribuição ao conhecimento na área temática estudada e propor diretrizes para novas pesquisas.

Agradecimentos

Caso for necessário, incluir-se-ão os agradecimentos ou reconhecimentos a pessoas, instituições, fundos ou bolsas de pesquisa que fizeram contribuições importantes na concepção, financiamento ou realização da pesquisa.

Literatura citada

Devem aparecer somente as referências bibliográficas mencionadas no texto. Não se aceitam notas de aula, artigos em construção ou no prelo, ou qualquer outra publicação de circulação limitada. Evitar o excesso de auto-citas.

A bibliografia deverá aparecer no final do texto, só com as referências citadas no mesmo. As citações no texto devem incluir sobrenomes do autor e ano, com vírgula entre autor e ano. Exemplo: Pérez, 1995; além de conservar a seguinte ordem de citação:

-Se houver mais de uma data, estas se separam com vírgula. Exemplo: Pérez, 1995, 1998, 2001.

-Se houver dois autores, estes se citam separados pela conjunção e. Exemplo: Gil e Ortega, 1993.

-Se houver vários trabalhos de um autor publicados no mesmo ano, estes se citam com uma letra em seqüência alfabética dos títulos, do lado do ano. Exemplo: Gómez, 2000a, 2000b, 2000c.

-Em caso de citações com três ou mais autores, é preciso mencionar no texto os sobrenomes do primeiro e substituir os outros pela expressão latina abreviada *et al.* que significa y outros; já na bibliografia devem aparecer citados todos os autores.

-As comunicações pessoais devem aparecer citadas no rodapé de página e não se incluem na bibliografia.

-As referências bibliográficas devem ir ordenadas alfabeticamente pelo sobrenome do primeiro autor, sem numeração e sem espaçamento na primeira linha. Para citar várias publicações do mesmo autor segue-se a ordem cronológica crescente, e no caso forem do mesmo ano seguirá a ordem alfabética dos títulos.

As referências deverão conter todos os dados que permitam sua fácil localização.

Exemplos:

Para livros: Autor(es), ano. Título do livro, edição, cidade de sua sede, casa editora e, páginas consultadas (pp. # - #) ou páginas totais (# p.). Exemplo: 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.

Para capítulos de livros: Autor(es), ano. Título do capítulo, páginas consultadas (pp. # - #). Em: Sobrenomes e nomes dos compiladores ou editores (eds.), título do livro, edição, casa editora e cidade de sua sede, páginas totais (# p.). Exemplo: Bernal H. 1996. Capítulo 6: Evapotranspiración. pp. 112-125. Em: Agrios G. (ed.). Fitopatología. Segunda edição. Editorial Limusa, México D.F. 400 p.

Para revistas: Autor(es), ano. Título do artigo, nome completo da revista (volume)número: página-página. Exemplo: 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. http://dx.doi.org/10.1093/treephys/24.4.38

Participações em memórias de congressos, seminários, simpósios: García M. 1998. La ingeniería geotécnica y la protección del medio ambiente. p. 65-94. Em: Memorias. IX Congreso Colombiano de la Ciencia del Suelo. Sociedad Colombiana de la Ciencia del Suelo. Santa Fé de Bogotá.

Teses, trabalhos de formatura. Gómez C. 2004. Autoecología del mortiño (*Vaccinium meriodinale* Swartz Ericaceae). Tese Mestrado em Bosques e Conservação Ambiental. Facultad de Ciencias Agropecuarias. Universidad Nacional de Colombia. Medellín. 78 p.

Abril G. 2002. Biogeografía y descripción de las especies del género *Collaria* sp. en seis zonas lecheras del Departamento de Antioquia, Trabalho de formatura. Facultad de Ciencias Agropecuarias. Universidad Nacional de Colombia. Medellín. 49 p.

Citação de citação. Magalhaes LM e da Cruz AJ. 1979. Fenologia do pau-rosa (*Aniba duckei* Kostermans), Lauraceae, em floresta primária na Amazônia Central. Acta Amazónica. 9(2): 227-232. Citado por: Gómez CP. 2004. Autoecología del mortiño (*Vaccinium meriodinale* Swartz Ericaceae). Tese Mestrado em Bosques e Conservação Ambiental. Facultad de Ciencias Agropecuarias, Universidad Nacional de Colombia. Medellín. 46 p.

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, Supl. 2: 195-201.

Para citas de internet: Autor(es), ano. Título do artigo. Em: Nome(s) da publicação eletrônica, da página web, portal ou página e sua URL, páginas consultadas (pp.#) ou páginas totais (# p.); data de consulta. Exemplo: Arafat Y. 1996. Siembra de olivos en el desierto palestino. Em: Agricultura Tropical, http://agrotropical. edunet.es. 25 p.; consulta: novembro 2003.

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.

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[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.

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[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.

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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.

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