Periodicidad: Cuatrimestral
Vol. 74 No. 2- 2021

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Dirección postal: Apartado Aéreo 568, Medellín, Colombia
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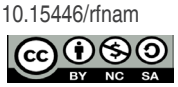
Página Web: http://www.revistas.unal.edu.co/index.php/refame
Teléfono: (*4) 430 90 06; Fax: (* 4) 230 04 20
Diagramación: Miryam Ospina Ocampo
Marcación: LandSoft S.A.
Diseño e Impresión: Centro de Publicaciones UN, Medellín.
Primera edición: Año 1939
ISSN: 0304-2847
ISSN formato web: 2248-7026
doi: 10.15446/rfnam



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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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EDITORIAL

La agricultura es la profesión del sabio, la más adecuada al sencillo y la ocupación más digna para todo hombre libre.

Cicerón

El tránsito de algunas ocupaciones humanas hacia *officĭum* aceptados y reconocidos se produce como consecuencia de los cambios que se presentan en las relaciones entre las sociedades y entre estas con la naturaleza. El reconocimiento social de las profesiones se da cuando ingresan a la escuela, el instituto, la facultad o la universidad, instituciones que le dan su aval académico. Las profesiones son entonces ocupaciones u oficios que requieren de conocimientos especializados, la capacitación educativa en diferentes niveles, control sobre el contenido de su ejercicio, autorregulación mediante la presencia de colegios de profesionales, sindicatos o gremios, el servicio a la sociedad y la existencia de normas éticas.

Con el surgimiento de las profesiones asociadas a la *Agricultura Científica,* las cuales eran diferentes a los *officĭum* generados por la *Agricultura Práctica*, se intentaba articular los desarrollos en las ciencias básicas (química, biología, botánica, zoología y genética) a la solución de los *problemas prácticos* de las actividades agropecuarias. La consolidación de las profesiones de la *Agricultura Científica* fue posible a la existencia de una sociedad que demandaba su presencia. Esa fue la intención que acompañó el nacimiento de varias profesiones que se crearon en Europa, la cual se aproximó a nuestro país con ritmos más lentos.

Más de 100 años después de su creación la Escuela de Agricultura Tropical y Veterinaria se transformó en la actual Facultad de Ciencias Agrarias. En esta centuria la Facultad modificó la denominación inicial del programa curricular por el de Ingeniería Agronómica, creó otros cinco programas de pregrado - Ingeniería Forestal, Zootecnia, Ingeniería Agrícola, Economía Agrícola y Tecnología Forestal – y varios de posgrado en el nivel de especialización (Ciencia y tecnología de alimentos, Gestión agroambiental, Nutrición animal, Sistemas de información geográfica), maestría (Bosques y conservación ambiental, Ciencias agrarias, Ciencia y tecnología de alimentos, Ingeniería agroindustrial) y doctorado (Ciencias agrarias, Ciencia y tecnología de alimentos y Ecología), trabajó en la generación de infraestructura de elevada calidad representada en cuatro estaciones agrarias (Cotové, Medellín, Paysandú, San Pablo), una Estación Forestal Experimental (Piedras Blancas), 29 laboratorios propios y otros que comparte con la Facultad de Ciencias, ha realizado numerosas reformas – algunas menores y otras estructurales – tanto en su programas curriculares como en sus procesos administrativos, ha garantizado una planta docente con la vinculación de tiempo completo y dedicación exclusiva de alta formación académica, cuenta con la *Revista Facultad Nacional de Agronomía,* la cual se mantiene en circulación desde 1939, como órgano de divulgación de la Facultad.

Al igual que en los inicios de la Escuela de Agricultura Tropical y Veterinaria, la Facultad de Ciencias Agrarias también enfrenta dificultades asociadas a la disponibilidad de recursos de financiación, declinación en el número de los estudiantes que procuran formación en su área de alcance, característica que no es exclusiva de ella, confrontación entre una enseñanza práctica para atender las demandas del mercado ocupacional con un horizonte muy cercano y la formación científica, creación de programas a veces con la misma raíz pero con otros enfoques y denominaciones y otras con características que buscan cubrir nuevos espacios, el cuestionamiento de numerosos sectores por las actividades agrarias, la intervención en los bosques y el uso de los recursos naturales y su desfavorable asociación con las profesiones del sector agrario y forestal.

A pesar de la complejidad de los problemas que acompañan a las actuales sociedades, los cuales harían necesaria la formación de profesionales en el campo agrario y forestal, se está en presencia de sociedades cuyos procesos culturales no conciben de la misma manera las necesidades de la agricultura concebida como uno de los principales proyectos humanos. Como aconteció en los anteriores 100 años la Facultad de Ciencias Agrarias dispone de las condiciones para realizar aquellas reformas estructurales que demanda el momento tanto con las reservas con las que cuenta en sus propios programas curriculares como con la creación de nuevas propuestas académicas.

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Review article

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Revista Facultad Nacional de Agronomía

Review on the ecophysiology of important Andean fruits: *Passiflora* L.

UNIVERSIDAD NACIONAL DE COLOMBIA

Revisión sobre la ecofisiología de frutos andinos importantes: *Passiflora* L.

https://doi.org/10.15446/rfnam.v74n2.91828

Gerhard Fischer[1]* and Diego Miranda[1]

ABSTRACT

The development of Andean fruit crops is viewed as an important and healthy contribution to global food consumption but ecophysiological studies on these fruit trees are scarce. 96% of approximately 520 *Passiflora* L. species are distributed in the Americas, especially in Colombia and Brazil. Many of these species originated on the edges of humid forests in tropical valleys. The four species: yellow passion fruit (*Passiflora edulis* f. *flavicarpa* Degener), sweet granadilla (*Passiflora ligularis* Juss.), purple passion fruit (*Passiflora edulis* f. *edulis* Sims) and banana passion fruit (*Passiflora tripartita* var. *mollissima* (Kunth) Holm-Niels & P.M. Jørg) are widely cultivated in Colombia, and their ecophysiological findings are described in this review. The demands, in terms of temperature (°C) and altitude (masl) are, for yellow passion fruits: 15-28 °C and 0-1,300 masl; sweet granadillas: 15-23 °C and 1,800-2,600 masl; purple passion fruits: 15-22/12-14 °C (day/night) and 1,600-2,300 masl; and banana passion fruit: 13-16 °C and 1.800-3.200 masl; all of them have high requirements for solar radiation, a minimum of 7 h of sunshine per day, to encourage flowering and fruit quality. Cloudy days decrease growth, flower bud induction and flower opening. Temperature and photosynthetic active radiation are the climatic factors that have the greatest effect on plant development. Relative humidity between 60 and 80% supports effective pollination and fecundation. *Passiflora* L. crops do not support long periods of waterlogging, with a maximum of 4 days for yellow passion fruit. Climatic events such as prolonged rain, intense droughts, strong winds and hail are harmful for these plants.

Keywords:
Altitude
Banana passion fruit
Purple passion fruit
Solar radiation
Sweet granadilla
Temperature
Yellow passion fruit

RESUMEN

El desarrollo de los cultivos frutales andinos se proyecta como una contribución importante y saludable para el consumo global de alimentos, pero los estudios ecofisiológicos de estos frutales son escasos. El 96% de aproximadamente 520 especies de *Passiflora* L. están distribuidas en las Américas, especialmente en los países Colombia y Brasil. Muchas de estas especies se originaron en los bordes de los bosques húmedos en los valles tropicales. Las cuatro especies: maracuyá (*Passiflora edulis* f. *flavicarpa*) granadilla (*P. ligularis*), gulupa (*P. edulis* f. *edulis*) y curuba (*P. tripartita* var. *mollissima*) son las más cultivadas en Colombia y de las cuales se describen hallazgos ecofisiológicos en esta revisión. Sus exigencias en temperatura (°C) y altitud (msnm) son para maracuyá: 15-28 °C y 0-1.300 msnm; granadilla: 15-23 °C y 1.800-2.600 msnm; gulupa: 15-22/12-14 °C (día/noche) y 1.600-2.300 msnm; curuba: 13-16 °C y 1.800-3.200 msnm, todas tienen altos requerimientos de radiación solar, necesitando mínimo 7 h de brillo solar por día lo que fomenta especialmente la floración y la calidad del fruto. Los días nublados disminuyen el crecimiento, la inducción de botones y apertura floral. La temperatura y la radiación fotosintéticamente activa son los factores climáticos que ejercen el efecto más grande sobre el desarrollo de las plantas. La humedad relativa entre 60 y 80% favorece una polinización y fecundación efectiva. Estos cultivos (*Passiflora* L.) no soportan periodos alargados de anegamiento, máximo por 4 días en maracuyá. Eventos climáticos como lluvia prolongada, sequías intensas, vientos fuertes y granizo son perjudiciales para estas plantas.

Palabras clave:
Altitud
Curuba
Gulupa
Radiación solar
Granadilla
Temperatura
Maracuyá

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Received: February 19, 2021; Accepted: April 01, 2021
Rev. Fac. Nac. Agron. Medellín 74(2): 9471-9481. 2021

ISSN 0304-2847 / e-ISSN 2248-7026

INTRODUCTION

Fruits of the tropics, with their production and supply throughout the year, open up a world of possibilities to expand crops and increase exports (Blancke, 2016). Nevertheless, much is lacking for proper cultivation and understanding their agroclimatic requirements (Fischer *et al*., 2016). Thus, the development of Andean fruit crops is seen as an essential and healthy contribution to global food consumption (Viera *et al*., 2019).

Many of these "exotic" fruits are classified as important functional foods (Moreno *et al*., 2014; Campos *et al*., 2018), not only in their countries of origin but also for populations in higher latitude regions (Ramadan, 2011), resulting in an excellent export opportunity for many Andean countries, which has been increasing in volume since the beginning of this century (Moreno-Miranda *et al*., 2019).

The Andean region, geographically, is a mountain range, with a length of about 8,500 km, from Chile to Venezuela, passing through countries as Argentina, Peru, Bolivia, Ecuador, and Colombia, with altitudes between 3,000 and 4,000 masl, surrounding the coastal zone of the Pacific Ocean (Guerrero *et al*., 2011). Its width ranges from 250 to 750 km and occupies an area of about 2'870,000 km^2 (Orme, 2007). The tropical Andes represent 15% of total global plant richness, standing out as a region with very high biodiversity (Peyre *et al*., 2019; Campos *et al*., 2018), and many fruit species originate in these South American areas (Ligarreto, 2012). Izquierdo and Roca (1998) characterized this "ecoregion" as one of the most fragile and misunderstood, where more than 60 million people live, half of them working in fields and many of them have very low income.

Ecophysiology studies examine environmental effects on plant physiology (Fischer *et al*., 2016), describing physiological mechanisms that interact with physical and biotic environmental factors during plant growth and development (Lambers *et al*., 2008). This information is of the utmost importance to achieve maximum production and quality on farms, which is only possible when environmental conditions are close to optimal for a species in order to benefit its genetic potential (Pérez and Melgarejo, 2015). Studies on environmental physiology have been widely used to improve species management or to recognize differences between different varieties (Restrepo-Díaz *et al*., 2010).

Factors such as temperature, solar radiation, altitude, rain, wind, and air pressure are the principal factors that influence these Andean crops (Fischer and Melgarejo, 2020; Restrepo-Díaz and Sánchez-Reinoso, 2020). On the other hand, the tropics do not have the marked temperature seasons seen in temperate zones; thus, the wet and dry seasons define the seasons to which plants react physiologically (Fischer and Parra-Coronado, 2020).

In nature, ecophysiological factors act holistically (Mittler, 2006), meaning studies under controlled environments with only one or two factors are questionable, and the concept of multidimensional ecophysiology makes it difficult to compare results obtained in different areas and countries (Fischer and Orduz-Rodríguez, 2012). As such, as Restrepo-Díaz and Sánchez-Reinoso (2020) stated, the results from recent years that are being used to improve fruit tree productivity need to be reevaluated considering the effects of climate change.

Climate change will strongly affect the high Andean tropics; on the one hand, it will increase precipitation by 20-25%, and, on the other, warming in the mountain region will be more intense than in the lowlands (Marengo *et al*., 2011). Rain not only predominates because of climate change in the high Andean zone, but precipitation is also the climatic factor that most affects the reproductive phase of plants (73.4%), compared to the influence of air temperature in this phase (19.3%), solar radiation and photoperiod (3.2%), as shown by a study on the phenology of plants in the Neotropics by Mendoza *et al*. (2017). The species more affected by climate change mainly include fruits and vegetables, for which Shukla *et al*. (2019) warned that production and quality will decrease as warming increases, especially in tropical and subtropical regions. Carr (2013) concluded that climate change and variability have impacted growing conditions in passion fruit production regions, which may lead to changes in productivity and commercial viability in these crops. Also, Posada and Ocampo-Pérez (2013) stated that the vulnerability of these crops to climate change will depend on their adaptation capacity in accordance with the genetic plasticity of the species (ecophysiology, genotype x environment). On the contrary, there are also indications that global warming

may increase production in fruit trees in some areas (Devenish and Gianella, 2012), and Tito *et al*. (2018) and Fischer and Melgarejo (2021) assume that a too high rise in the crops growing temperature could be avoided in the Andes by using higher altitude areas (with lower temperatures).

The development of many promising crops that have great potential in international markets is affected by a lack of knowledge on climate impacts on growth, production, and quality (Fischer *et al*., 2009). There are few studies on ecophysiology in *Passiflora* crops despite the fact that Ocampo (2013) highlighted its importance given that much of the knowledge comes from the observations of fruit growers who cultivate them in different areas of the country (Pérez and Melgarejo, 2015).

Therefore, this article aimed to collect the information on the ecophysiology of passion fruit species cultivated in the Andean region, with an emphasis in Colombia, in order to understand the climatic requirements of each crop and the effects on their physiological processes, which are key to adaptation, management and improvement of the crop.

Some ecophysiological characteristics of the higher altitude tropics

The Andean region offers growing conditions for numerous fruit species that may thrive up to 3,000 masl or more, whether they are frost-resistant crops or avoid frost during the flowering season or in recently set fruits (Fischer and Orduz-Rodríguez, 2012). For instance, in Colombia frost does not occur below 2,400 masl, and banana passion fruits are found up to an altitude of 3,200 masl (Angulo and Fischer, 1999). Solar radiation in the highlands encourages the thickening of the fruit epidermis and a greater number of parenchyma layers, and the high ultraviolet (UV) radiation results in a greater formation of antioxidants, factors that favor the quality of these fruits (Fischer *et al*., 2016; Fischer, 2000). Therefore, trellis systems (*sistema de conducción* or *emparrado*) are recommended in areas with excessive radiation (Fischer *et al*., 2009).

As thermal altitude classification confirms, temperature is the most influential climatic factor, which changes as altitude increases, with a decrease of about 0.6 °C per 100 m of elevation, mainly affecting the duration of growth cycles and the phenology of fruit trees (Fischer *et al*., 2009). Likewise, with an increase in altitude, the partial pressure of gases, such as O_2, CO_2 and N_2, and air humidity decrease, along with a reduction in precipitation, from the altitude of 1,300-1,500 m (Fischer and Orduz-Rodríguez, 2012). On the contrary, these authors indicated that ultraviolet, visible, and infrared radiation increase with altitude, along with wind speed.

The leaf thickness increases, and the leaves become smaller when exposed to the UV light, which may be due to the stress caused by increasing altitude (Fischer *et al*., 2016). In addition, UV radiation affects the production of auxins (Fischer and Melgarejo, 2014). Buchanan *et al.* (2015) stated that UV light causes a lower synthesis of gibberellins in internodes, which means that high- altitude fruit trees reduce the longitudinal growth of the stem, compared to plants in the lower areas.

Passifloraceae

Rodríguez *et al.* (2020a) and Ocampo *et al.* (2021) reported that South America is the center of diversity for most cultivated *Passiflora* species. The high biodiversity of *Passiflora* in South America puts the continent as a region of great potential for utilization of these species (Hurtado-Salazar *et al*., 2021). There are about 520 species of passion flowers, and approximately 96% of these are distributed in the Americas, being Colombia and Brazil the centers of the diversity with 30% (Cerqueira-Silva *et al*., 2014). These species include herbaceous and woody vines, usually with tendrils, and a few are shrubs or trees (Ocampo *et al*., 2021). The species of this family are native to tropical and subtropical regions of both hemispheres and grow in low- and high-altitude habitats, where temperatures are moderate (Paull and Duarte, 2012). Ocampo *et al.* (2007) stated that the passion fruit plants are originated in the edges of humid forests in tropical valleys, with 20 to 30 °C average air temperature and high environmental humidity and precipitation rates but these conditions does not appear, in all cases, to be optimal for growth and production (Hurtado-Salazar *et al*., 2021). These are semi-perennial fruit-bearing species of a climbing habit (Primot *et al*., 2005).

This family, given its permanent production in the interior tropics because of its indeterminate growth habit, requires well-distributed rainfall throughout the year (Fischer *et al*., 2018). Jackson *et al*. (2010)

recommended areas that are not too cold for these species, with high solar brightness and winds that are not strong. However, as these authors pointed out, regions with very hot summers decrease crop longevity, and frost below -2 °C causes severe damage to plants.

Colombia has the greatest diversity of passion fruit species (Ocampo *et al*., 2007), where nine species are cultivated to meet the needs of local markets and export demands (Ligarreto, 2012), with greater production in the departments of Antioquia and Huila, especially for yellow passion fruit, sweet granadilla and gulupa (Fischer *et al*., 2018). The species requirements for altitude and temperature vary widely. The banana passion fruit plants are well-adapted to the cold climate (Table 1).

Table 1. Origin of four important passion fruit trees cultivated in the Andean region.

Species	Zones and countries of origin	Authors
Yellow passion fruit	Amazon region	Blancke, 2016
Sweet granadilla	From Bolivia to Central America	Blancke, 2016
Purple passion fruit	South of Brazil, Paraguay, and North of Argentina	Ocampo *et al*., 2020
Banana passion fruit	Mountainous area of South America (Colombia, Ecuador, Venezuela, Bolivia, Peru)	Fischer *et al*., 2020

In addition to these four commercially important passion fruits, Colombia has other species of great importance, such as giant granadilla "badea" *(P. quadrangularis*) and stone granadilla "cholupa" (*P. maliformis*) (Rodríguez *et al*., 2020b).

Yellow passion fruit

The most cultivated and well-known passion fruit species is the yellow passion fruit (*Passiflora edulis* f. *flavicarpa* Degener), also known as acid passion fruit; it is a product of great commercial and economic importance in Brazil, thanks to the quality of its fruits and its high industrial yield (Faleiro *et al*., 2020). It can be cultivated between 0-1,300 masl (Table 1), according to Fischer *et al*. (2009), it is the most tropical passion fruit with better adaptation to the lower marginal zone of the coffee region in Colombia.

The yellow passion fruit grows optimally in temperatures between 23 and 25 °C, while in regions with temperatures below 15 °C and a photoperiod with less than 11 h light per day, there is little flowering stimulation (Faleiro *et al*., 2020) (Table 2). Regions with average temperatures higher than 28 °C, accelerate vegetative growth but with a reduction in production because of dehydration of the stigmatic fluid (Fischer *et al*., 2009). Cleves (2012) recommended a minimum of 5 h of direct sunlight on plantations and highlighted that fruits directly exposed to the sun have a higher concentration of ascorbic acid and total soluble solids, with a thinner rind, however, growers always have to avoid sunburn damage**.**

Relative humidity below 30% reduces pollination and fruit development (Faleiro *et al*., 2020); these very dry conditions inhibit photosynthesis by closing stomata and burning the tender shoots of plants (Fischer *et al*., 2009). Faria *et al*. (2020) irrigated yellow passion fruit seedlings with 25, 50, 75 and 100% of the capacity of the pot substrate, and found that, in 50%, the plant height and the stem diameter were very similar to those plants with greater amounts of water (up to 175%) and stated that the plants possibly have water reserves in the stem and roots. Likewise, these authors observed that the number of leaves, the transpiration and the biomass of the plants constantly increased up to 100% of the capacity of the pot substrate.

Yellow passion fruit plants do not tolerate waterlogging for more than four days, which especially affects roots and stem growth (Basso *et al*., 2019). As shown in Table 3, flooded yellow passion fruits increase the relative water content, but the photosynthetic and transpiration rates

Table 2. Climatic factors for the growth and production of passion fruits in Colombia (modified according to Fischer *et al*., 2018)

Climate factor	Yellow passion fruit (*P. edulis* f. *flavicarpa*)	Sweet granadilla (*P. ligularis*)	Purple passion fruit (*P. edulis* f. *edulis*)	Banana passion fruit (*P. tripartita* var. *mollissima*)
Temperature (°C)	15-28 (opt. 23-25)	15-23 (opt. 18-20)	15-22 (opt.15-18)	13-16 (opt. 12-16)
Altitude (masl)	0-1,300	1,800-2,600	1,600-2,300	1,800-3,200
Precipitation (mm year^{-1})	800-1,500	1,500-2,500	1,800-2,300	1,000-1,500
Relative humidity (%)	70	60-80	60-70	70-80
Solar radiation	>11 h sunshine per day	1,500-1,600 h sunshine per year	7-9 h sunshine per day	7-8 h sunshine per day
Authors	Cleves *et al*., 2012; Faleiro *et al*., 2020	Miranda, 2020	Ocampo and Posada, 2012; Ocampo *et al*., 2020	Campos and Quintero, 2012; Fischer *et al*., 2020

decrease, along with the stomatal conductance (Govêa *et al*., 2018; Faria *et al*., 2020). Nonetheless, because of the formation of an aerenchyma in the cortex of flooded plants (Govêa *et al*., 2018), passion fruits have mechanisms of adaptation to higher humidity conditions in the root environment for a time; thus, Faria *et al*. (2020) classified this fruit as moderately sensitive to water excess in the soil.

Table 3. Effect of waterlogging on the physiological response of yellow and purple passion fruits.

Crop	Physiological response to waterlogging	Reference
Yellow passion fruit	- Plants tolerate waterlogging up to 4 days. - Root and stem biomass were mainly affected. - With increased waterlogging, the hydration of the plant increased. - Waterlogging of ≥5 days caused irreversible negative effects on the plant.	Basso *et al.* (2019)
Yellow passion fruit	In plants watered for 22 days with 100, 125, 150 and 175% of the capacity of the pot substrate: - The height of the plant and the diameter of the stem were not affected, while the biomass was reduced. - The transpiration was reduced with the increase of the water starting at118% of the substrate capacity. - The relative water content of the plant increased with irrigation.	Faria *et al.* (2020)
Yellow passion fruit	In seedlings kept with soil moisture at field capacity, soil pre-immersed in water or flooded, for 7 days: - Waterlogging reduced the photosynthetic rate, stomatal conductance and intracellular CO_2. - There were no differences in the water potential or the proline content of the leaves. - Plants in pre-submerged and flooded soil increased the diameter of roots, epidermis, cortex and endodermis of the root system, also forming an aerenchyma.	Govêa *et al.* (2018)
Purple passion fruit	- Mycorrhized seedlings tolerated waterlogging better because of higher leaf retention, proline and chlorophyll production, but lower synthesis of carotenoids and total soluble sugars. - In non-mycorrhized plants, the nitrogen and phosphorus contents in the leaves were reduced faster. - Waterlogging reduced mycorrhizal colonization of the roots.	Chebet *et al.* (2020)

Sweet granadilla

In Colombia, the sweet granadilla crop (*Passiflora ligularis* Juss.) is the second one in species of the genus *Passiflora* because of its economic importance (Ocampo *et al*., 2015). It adapts well to elevations between 1,800 and 2,600 masl, with optimal temperatures between 18 and 20 °C (Table 1) (Miranda, 2020). Temperatures higher than 23 °C cause thermal stress, and those between 15 and 18 °C increase the duration of the production cycle but decrease the productivity (Miranda, 2012). As this author affirmed, there are problems with temperatures below this range, namely those between 12 and 15 °C, which increase the abortion of flowers, reduce fecundation, and cause cracking of recent set fruits. Rivera *et al.* (2002) reported that below 1,800 masl, the incidence of insect-pests increases, and smaller fruits develop. Also, below 1,500 masl, the viability of pollen is low.

In the municipality of Santa María, Department of Huila (Colombia), Fernández *et al.* (2014) ecophysiologically characterized sweet granadilla *in situ* at 2,060 masl (average temperature 17.15 °C, PAR (photosynthetic active radiation) 1,186.2 µmol photons m^{-2} s^{-1}) and at 2,270 masl (16.24 °C, PAR 470.9 µmol photons m^{-2} s^{-1}). At 2,060 masl, the maximum photosynthetic rate (A_{max}) 23.6 µmol m^{-2} s^{-1} of CO_2, the darkness respiration rate (DR) 2.24 µmol m^{-2} s^{-1} of CO_2 , and the compensation point per light (I_c) 34.6 µmol m^{-2} s^{-1}, were higher than at 2,270 masl (17.5 A_{max}; 1.34 DR and an I_c of 21, respectively), while for the two locations, the foliar water potential (about -0.2 MPa), the edaphic one (about -0.01 MPa) and the predawn values of maximum photochemical efficiency of photosystem II (Fv/Fm; >0.86) indicated that the plants did not suffer any stress, concluding that both sites are suitable for the commercial cultivation of sweet granadilla (Fernández *et al*., 2014).

Among the physiological disorders of sweet granadilla, sunburn is the most important physiopathy, caused by excess solar radiation in fruits unprotected by leaves, accentuated after pruning of branches in the fruitful part of the plant or by a drought that also reduces the leaf area (Rivera *et al*., 2002). Fruit cracking is a physiopathy related to sudden changes in day to night temperature but can also be caused by a high Ca deficiency (Miranda, 2020), irrigation or a heavy rain after a prolonged dry season that increases the senescence of the epidermis and its extensibility (Fischer and Orduz-Rodríguez, 2012).

Regarding the water factor, a commercial sweet granadilla plantation requires rainfall between 1,500 and 2,500 mm $year^{-1}$ and an atmospheric humidity of 60 to 80%, which favors the activity of pollinators, while higher humidity increases the incidence of fungal pathogens (Rivera *et al*., 2002; Miranda, 2020). As in other passion fruit species, water stress in the reproductive stages, from the pre-flowering stage to the filling of the fruit, causes the abscission of the floral structures and considerably reduces yield (Miranda, 2020). Likewise, this detrimental effect in the reproductive phase is related to the deficiency of nutrients, such as P, K, Ca and B. Under a moderate water stress in sweet granadilla plants generated a decrease in leaf area, number of leaves and longitudinal growth of branches, while the root/aerial ratio of the plants increased with the duration of water stress (Casierra-Posada and Roa, 2006).

According to Table 2, the light in a technical cultivation of sweet granadilla requires 1,500 to 1,600 h $year^{-1}$ of sunshine (Miranda, 2020) because this luminosity highly encourages the differentiation of floral primordia, flowering and fruit coloration (Miranda, 2009).

On the other hand, excessive winds are detrimental to this passion fruit species since they affect pollination agents, bees and bumblebees, and can damage flowers and pollen germination as a result of drying of the stigmatic surface, while calm environments ensure better fruit set (Miranda, 2009).

Purple passion fruit

While Blancke (2016) confirmed that the purple passion fruit (*Passiflora edulis* f. *edulis*) is cultivated not only in the subtropics but also in high Andean areas, in Colombia, Ocampo *et al.* (2020) reported that crops are found from 1,600 to 2,300 masl, avoiding very steep slopes, with the best results in terms of production and quality found in areas from 1,700 to 2,000 masl. Ocampo and Posada (2012) specified this as the optimal range to develop good genetic vigor, good pollination and fecundation, and low incidence of pests and diseases. Interestingly, Rodríguez *et al.* (2019), who studied 50

purple passion fruit genotypes at two different altitudinal locations, Pasca (Cundinamerca, 1,800 masl, 18 °C) and Susacón (Boyacá, 2,500 masl, 13.1 °C), found that the 34 landraces had a better adaptive physiological response at the higher altitude than the commercial genotypes, which is because they are more suitable for plant breeding programs in the current climate change scenario. Carr (2013) observed that the purple passion fruit can be cultivated up to 3,000 masl in the tropics.

Optimal temperatures for purple passion fruits are in the range of 15-22 °C day and 12-14 °C night (Table 2) (Pérez and Melgarejo, 2012), tolerating temperatures ranging between 10 and 24 °C (Ocampo *et al*., 2020). In their ecophysiological study at three different locations in Cundinamarca (Colombia), Pérez and Melgarejo (2015) concluded that climatic conditions similar to those in the municipality of Granada (2,230 masl, 15 °C), which has a day/night temperature of 18/13 °C, a vapor pressure deficit close to 0.05 kPa and radiation that does not exceed 1,000 µmol photons m^{-2} s^{-1}, greatly favor the physiological performance of purple passion fruits. Temperatures over 30/25 °C day/night affect flower production (Jiménez *et al*., 2012); adult plants resist low temperatures but can be damaged by temperatures between -1 and -2 °C (Paull and Duarte, 2012).

Sunlight from 7 to 9 h day^{-1} favors fruit quality (Pérez and Melgarejo, 2012), while Ocampo and Posada (2012) stated that excessive cloudiness during fruiting delays ripening, and decreases the content of soluble solids (° Brix) and the quality of the juice. The purple passion fruit is very susceptible to changes in solar radiation because they affect the productivity of plants; thus, cloudy days decrease growth, induction of flower buds and flower opening. A reduction of light of 1-4 weeks decreases flowering and plant production. Besides, an excess radiation generates sunburning on fruits and decreases normal plant development (Jiménez *et al*., 2012).

Ocampo *et al.* (2020) recommended relative humidity (RH) between 60 and 70% for cultivation, which sustains effective pollination and fecundation, so the stigmas remain hydrated and adhesive. Rain during flowering affects the functionality of pollen because it can burst and cause abscission of flowers (Jiménez *et al*., 2012). Carr (2013) reported that rain up to 2 h after pollination prevents fruit set. Sánchez *et al.* (2013) found a negative correlation between RH and stomatal opening on a purple passion fruit plantation, but positive correlations between solar radiation and temperature with stomatal opening.

According to Ocampo *et al.* (2020), well-distributed rainfall between 1,800 and 2,300 mm $year^{-1}$, is favorable for the growth and production of the purple passion fruit, while for the reproductive phases, i.e. between the sprouting of flower buds and fruit enlargement, water stress causes very small fruits or aborted fruits (Jiménez *et al*., 2012). Paull and Duarte (2012) reported that a water stress less than -1.3 MPa leads to a strong reduction in the development of the foliar area, flowering and plant yield, pointing out that the lack of water can be one of the environmental factors responsible for production fluctuations in these passion fruits.

In another purple passion fruit maypop (*P. incarnata*), García-Castro *et al.* (2017) found a moderate tolerance to short water deficit periods because of its stomatal mechanism and other non-stomatal factors (up to 10% of evapotranspiration, ET), with an exponential reduction in the photosynthetic rate with a water stress more negative than -1.0 MPa; however, the plants quickly recovered their gas exchange properties when they were irrigated again at 100% ET. Also, Crane *et al.* (2019) classified passion fruits as moderately water-stress tolerant plants that can withstand this stress for several days but with reduced plant growth and yield. Carr (2013) observed that the production of new leaves ceases at a leaf water potential -2.0 MPa, and the expansion of new leaves is considerably reduced to -1.5 MPa in *P. edulis*.

In general, the purple passion fruit, like the other passion fruits, does not resist periods of waterlogging (Crane *et al*., 2019); however, Chebet *et al.* (2020) found that mycorrhized plants (with a mixture of *Glomus caledonium*, *G. etunicatum*, *Gigaspora magarita* and *Scutellospora* sp.) better tolerate this adversity, producing more osmoprotectant proline and chlorophyll and thus, retaining their leaves and foliar N and P for longer than non-mycorrhized ones (Table 3).

Strong winds affect pollinating insects, which do not fly at high wind speeds (Fischer and Orduz-Rodríguez,

2012) and can damage flower structures and dehydrate pollen and stigma, along with damage to plantations and conduction systems (Ocampo *et al*., 2020).

Banana passion fruit

The banana passion fruit (*Passiflora mollissima* f. *tripartita*) is favored by altitude because UV radiation promotes the thickness of the epidermis, increasing resistance to diseases, mainly anthracnose (Campos and Quintero, 2012). There are commercial plantations between 1,800 and 3,200 masl in Colombia (Fischer *et al*., 2020), with the best conditions between 2,000 and 3,000 masl (Angulo, 2003). In countries such as Bolivia and Peru, there is cultivation up to 3,300 and 3,400 masl (Blancke, 2016; National Research Council, 1989). Mayorga *et al*. (2020) studied banana passion fruits at altitudes of 2,498 and 2,006 m in Pasca (Cundinamarca, Colombia), observing that, in the higher altitude, larger fruits were formed, with higher levels of citric and ascorbic acid but with less total soluble solids (°Brix), and recorded temperature and photosynthetic active radiation (PAR) as the climatic factors that exerted the greatest effect on plant development. To resist large fluctuations in temperature and high radiation (UV) in high Andean zones, plants develop soft hairs on the entire vegetative part (Angulo and Fischer, 1999). Sunlight between 1,300 and 1,600 h year^{-1} are necessary to guarantee favorable growth and production conditions for plants (Angulo, 2003).

This species is the commercial passion fruit most adapted to cold tropical and subtropical regions (Blancke, 2016). Mayorga *et al*. (2020) established the base (minimum) temperatures for the growth of primary branches, flower buds and fruits as 4.3, 3.1 and 0.01 °C, respectively. Zones with temperatures between 13 and 16 °C are recommended for cultivation, but temperatures below 0 °C can affect flower buds, flowers, fruit set and non-lignified vegetative parts of the plant (Campos and Quintero, 2012). Given that this fruit grows at altitudes higher than the frost limit, *P. mollissima* f. *tripartita* lines have formed tolerance to temperatures of -5 °C for a short time (National Research Council, 1989).

Rainfall of 1,000 to 1,500 mm year^{-1}, well-distributed throughout the year, is optimal for commercial crops (Fischer *et al*., 2009); therefore, supplying moisture in dry periods with artificial irrigation is necessary, especially in the reproductive phases. Climatic events such as prolonged rain (waterlogging), intense droughts and hail can affect the cultivation and production of plants (Fischer *et al*., 2020). Campos and Quintero (2012) recommended a relative humidity between 70 to 80%, which favors pollination and pollen germination; considering that drier airs increase the risk of frost in the high Andean zone, while a higher atmospheric humidity benefits some diseases, especially anthracnose. Light winds facilitate the transport of pollen through the air and the flight of pollinating insects, which are necessary since the banana passion fruit is an allogamous plant. Strong winds must be controlled by planting windbreak barriers using native plants (Fischer *et al*., 2020) that also protect against flower fall (Angulo, 2003).

The cultivation of passion fruit plants in Colombia reveals a positive impact of plastic roofing oriented over the plant rows with a PE plastic (175 µm thickness) 1.50 m wide, which is placed on a “T” type structure located at 2.2 m above the ground. This structure protects the plants from heavy rains and direct solar radiation and regulates the circulation of air inside the canopy, reducing relative humidity. These factors improve the production and quality of the fruits and contribute to reduce the incidence of critical fungal diseases, such as *Alternaria* sp. and *Cladosporium* sp. Nevertheless, the use of this technique is still scarcely reported since it is necessary to carry out physiological studies of these crops that explain the variables of yield and quality of the fruit production.

CONCLUSIONS

The temperature requirements for the studied passion fruit species are, generally, between 15 and 23 °C; only the banana passion fruit requires a temperature range between 13 and 16 °C. For altitude requirements, the yellow passion fruit is the most “tropical” species, growing at the elevations between 0 and 1,300 masl, and the banana passion fruit is the most “Andean” one, which is found between 1,800 and 3,200 masl.

Sufficient solar radiation and PAR are crucial for flower induction and flowering, while a relative humidity between 60 and 80% favors pollination and fecundation. Sweet granadilla and purple passion fruit plants require

precipitations rates between 2,300-2,500 mm year^{-1}. On the other hand, a new technology consisting in the plastic cover placed over the rows of purple passion fruit plants protects the plants against heavy rains and the fruits against direct sunlight. This technology allows for a lower incidence of pests and diseases and, therefore, provides a higher fruit quality and production; however, there is a lack of studies to understand better these beneficial effects.

These crops are moderately sensitive to waterlogging, having a resistance mechanism of aerenchyma and, on the other hand, the mycorrhizal plants better tolerate this abiotic stress. The passion fruit plants withstand a moderate water shortage; however, very dry conditions at reproductive stages, starting from the pre-flowering up to the fruit filling, cause the abscission of the floral structures and can considerably reduce the yield.

In addition to hailstorms, strong winds are very harmful to *Passiflora* plants, since affect the activity of pollinating insects and can damage flower structures and dehydrate pollen and stigma along with the damage produced to plantations and conduction systems.

The current climatic variability affects the physiology of plants and orchards and requires the implementation of new agronomic practices for special management of the temperature and precipitation effects.

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Revista Facultad Nacional de Agronomía

UNIVERSIDAD NACIONAL DE COLOMBIA

Development of lettuce varieties in different organic wastes as substrate

Desarrollo de variedades de lechuga en diferentes residuos orgánicos como substrato

https://doi.org/10.15446/rfnam.v74n2.85547

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ABSTRACT

To improve crop development, commercial substrates are recommended without distinction for different crops and/or varieties without considering their characteristics and needs; therefore, their composition and nutritional condition must be studied for each type of plant in its initial formation. The objective of this study was to evaluate the development of shoot and root systems of two lettuce cultivars produced in 10 different substrates. Great Lakes and Simpson Black Seed cultivars were evaluated in 10 substrates formulated by mixing a commercial substrate, organic compounds (swine, cattle, poultry) and sugarcane bagasse. Great Lakes cultivar had a higher development of the aerial part, whereas Simpson Black Seed cultivar had a more robust root system. The substrate with swine and poultry favored the development of the aerial part of the seedlings and lettuce, while the substrates with 33% of sugarcane bagasse improved the development of the root system. The commercial substrate used in isolation showed the lowest performance.

Keywords:
Great Lake
Lactuca sativa L
Organic compost
Wastes

RESUMEN

Para mejorar el desarrollo de cultivos, se recomiendan sustratos comerciales indistintamente del tipo de cultivo y/o variedad sin considerar sus características y necesidades. Por lo tanto, se debe estudiar su composición y condición nutricional para cada tipo de planta en su etapa inicial. El objetivo de este estudio fue evaluar el desarrollo del sistema de brotes y raíces de dos cultivares de lechuga producidos en 10 sustratos diferentes. Los cultivares Great Lakes y Simpson Black Seed se evaluaron en 10 sustratos formulados mezclando un sustrato comercial, compuestos orgánicos (cerdos, ganado y aves de corral) y bagazo de caña de azúcar. El cultivar Great Lakes tuvo un mayor desarrollo de la parte aérea, mientras que el cultivar Simpson Black Seed tuvo un sistema de raíces más robusto. El sustrato que contenía los residuos provenientes de cerdos y aves fue el que favoreció el desarrollo de la parte aérea de las plántulas y la lechuga, mientras que los que contenían el 33% del bagazo de caña de azúcar fueron los que favorecieron el desarrollo del sistema radicular. El sustrato comercial utilizado de forma aislada, mostró el rendimiento más bajo.

Palabras clave:
Great Lake
Lactuca sativa L
Compuesto orgánico
Residuos

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Received: April 29, 2020; Accepted: December 4, 2020
Rev. Fac. Nac. Agron. Medellín 74(2): 9483-9489. 2021

ISSN 0304-2847 / e-ISSN 2248-7026

Lettuce (*Lactuca sativa*) is a leafy vegetable belonging to the Asteraceae family. This vegetable is widely cultivated throughout Brazil, being an integral part of the great importance of the Brazilian diet (Antunes *et al*., 2016). This crop has an excellent adaptation to the climate of different Brazilian regions, low cost of production and presents possibility of growing all year in most of the country. Besides being very easy to market, it has a great economic and social importance (Medeiros *et al*., 2008).

Several factors influence the crop productivity, such as edaphoclimatic conditions and genetic factors related to cultivars and their adaptation to specific environmental conditions. In regions with high temperatures and high luminosity, the choice of cultivars that are not erect and tolerant because in such situations, there is a difficulty in forming the head and anticipation of the reproductive period, promoting the loss of quality and productivity (Souza *et al.,* 2008). About 60% of a crop's success lies in planting good quality seedlings (Luz *et al.,* 2010). The system of multicellular trays is the most used in the production of vegetable seedlings. Tomato seedlings, lettuce, cabbage, cauliflower, sweet pepper, and eggplant are currently produced in this system, using commercial substrates or substrates made by the producers composting organic waste (Marques *et al*., 2003).

In addition to the need to produce high quality seedlings, horticulturalists have the need to reduce the costs of the activity (Medeiros *et al*., 2008). One of the alternatives that should reduce costs associated with the production of quality seedlings is the use of organic compounds available in the production region. The intensive use of agricultural soils, specifically those originating from horticultural production, causes a decrease in organic matter and nutrients. Many authors such as Giannakis *et al.* (2014) and Pellejero *et al.* (2017) point out that the application of organic compounds in the soil has positive effects on the nutritional properties and on the quality of the harvested fruits.

A suitable substrate should not contain soil because of the presence of pathogens and weed seeds; besides, the seedling removal from the container with the whole lump is more complicated to handle (Boaro, 2013). A quality substrate must possess physical, chemical, and biological characteristics that provide adequate conditions for good germination, and that favors the development of the seedlings (Andrino, 2018), allowing a good development of roots and shoot. The properties usually used for the characterization of a substrate are pH, cation exchange capacity, salinity and organic matter content (Schmitz *et al*., 2002). For the chemical characteristics of a substrate, the pH must be in the appropriate range for the cultivation of the plant. Electrical conductivity is related to the availability of nutrients; it refers to the concentration of ionized salts in the solution (Kratz, 2015). Among the most important physical properties, the density of the substrate, the total porosity, the aeration space and the water holding capacity stand out. The biological characteristics are linked to the presence of pathogenic agents, such as nematodes, mites and phytopathogenic microorganisms, which can harm the development of the plant (Takane *et al*., 2012). One only material cannot bring together all the characteristics appropriate to the needs of the plants, therefore, it is a common practice to use mixtures to obtain the desired properties (Damiani and Schuch, 2009). In this context, the aim of this work was to evaluate the development of two lettuce cultivars produced using 10 different substrates.

MATERIALS AND METHODS

The experiment was conducted from March to April 2014 in a greenhouse located at the State University of Goias campus Santa Helena de Goias, Goias, Brazil, at coordinates 17°49'23''S 50°35'18''W. The region's climate is tropical, with rains concentrated in summer (October to April) and a well-defined dry period during winter season (May to September). The average annual temperature ranges 20 °C to 35 °C. A randomized block experimental design was used with a factorial scheme 2x10, with four replicates in duplicate (Table 1). The factors consisted of two lettuce cultivars: Great Lake (from the American group) and Simpson Black Seed (from the Crespay group) and 10 substrates.

Sowing was carried out in tubes with a volume of 127 cm^3 (140 mm height, 37 mm diameter of the upper hole (without flap), 47 mm diameter of the upper hole (with flap) and 12 mm diameter of the lower hole. Cattle,

poultry and pig residues were subjected to composting and then dried outdoor. After drying the residues, they were sieved to obtain a fine and homogeneous fraction, except the sugarcane bagasse and the commercial substrate Plantmax®, as these were already in ideal conditions to use.

Table 1. Composition of the substrates used in the study.

S1	100% Plantmax® commercial substrate
S2	50% Plantmax® + 50% swine waste
S3	50% Plantmax® + 50% bovine waste
S4	50% Plantmax® + 50% poultry litter
S5	50% Plantmax® + 50% sugarcane bagasse
S6	33% Plantmax® + 33% sugarcane bagasse + 33% poultry waste
S7	33% Plantmax® + 33% sugarcane bagasse + 33% bovine waste
S8	33% Sugarcane bagasse + 33% pig waste + 33% bovine waste
S9	33% Plantmax® + 33% sugar cane bagasse + 33% pig waste
S10	33% Bird waste + 33% pig waste + 33% bovine waste

After sowing, the tubes were kept in the greenhouse, and the evaluations were performed 30 days after sowing. Evaluated parameters:

- Number of leaves (NL): it was counted in each plant.

- Plant height (PH): it was determined from the base of the stem to the apex of the aerial part.

- Fresh mass of the aerial part (FAM) and root (FRM): to obtain the fresh mass of the aerial part, plants were weighed by a precision scale right after the removal of the seedlings from the tubes. The same procedure was performed for the root system.

- Dry mass of the aerial (DMA) and root (DRM): to obtain the dry mass, the weighed plants were used to obtain the weight of the fresh mass, and immediately afterwards, they were stored in an oven with forced air circulation at 65 ° C for 72 h to dry, until they reached constant mass.

The data were subjected to analysis of variance by the F test at 5% significance and the tukey test was applied to means using the Sisvar software.

RESULTS AND DISCUSSION

The interaction between the two evaluated factors (cultivars x substrates) affected the variables FAM (Table 2) DRM (Table 2). Simpson cultivar planted in the substrate S6-(33% Plantmax®+33% sugarcane bagasse+33% poultry litter) was higher than Great Lakes in this same substrate for the FAM variable (Table 2).

Evaluating the FAM, the substrate S8 showed the best result for the Great Lake cultivar and the substrate S6 for the Simpson Black Seed cultivar, which allowed a greater formation of the aerial part of the plants during the seedling production phase, considering the leaves as a great source of assimilates for the other organs of the plants. Higher production of FAM part can result in better development of the seedlings after transplantation, since the seedlings with more developed aerial part have a greater capacity to withstand the stress caused by this process. According to Bellote and Silva (2010), leaves are one of the main sources of photoassimilates (sugars, amino acids and hormones) and nutrients for adaptation of post-planting seedlings, which require a good reserve of photoassimilates (water and nutrients for the roots).

Table 2. Fresh mass of the aerial part (FAM) and dry mass of the aerial (DMA) regarding lettuce cultivars and 10 substrates.

Substrates	FAM (g)		DMA (g)	
	Great Lake	Simpson Black Seed	Great lake	Simpson Black Seed
S1	0.78 d A	0.985 f A	0.100 d A	0.098 e A
S2	9.048 ab A	9.518 ab A	0.288 abc A	0.330 bc A
S3	3.465 cd A	2.755 ef A	0.163 cd A	0.113 de A
S4	9.215 ab A	11.178 ab A	0.173 cd A	0.280 bcd A
S5	5.948 cd A	5.113 de A	0.395 a	0.318 bc A
S6	7.718 ab B	11.658 a	0.185 bcd B	0.688 a
S7	6.528 abc A	5.993 cde A	0.433 a A	0.320 bc B
S8	9.405 a A	8.593 abc A	0.365 ab A	0.355 b A
S9	6.935 ab A	8.150 bcd A	0.213 bcd B	0.373 b A
S10	5.905 bc A	5.880 cde A	0.193 bcd A	0.163 cde A
MSD*	3.436		0.182	
Average Overall	6.733		0.277	
Default Error	0.739		0.039	
CV (%)	21.96		28.19	

Means followed by the same lower case letter in the columns or upper case in the rows do not differ from each other by the Tukey test at 5% significance. *Minimum Significant Difference

The two studied cultivars influenced the PH and NL variables; however, no differences were observed for the interaction between them (Table 3). The Great Lakes showed better results than the Simpson Black Seed cultivar in the NL and PH. These variables referring to the aerial part, where Simpson Black Seed cultivar was higher than the FRM, demonstrating that under the experiment conditions, the Great Lake cultivar presented a higher development of the aerial part, while the Simpson Black Seed cultivar had a more robust root system.

Table 3. Number of leaves (NL), plant height (PH) and fresh mass of the root (FRM) as function of two cultivars.

Cultivars	NL	PH (cm)	FRM (g)
Great Lake	6.300 a	12.984 a	1.580 b
Simpson Black Seed	5.838 b	11.710 b	1.786 a
MSD*	0.24	0.87	0.21
Average Overall	6.07	12.35	1.68
Default Error	0.086	0.31	0.72
CV (%)	8.95	15.76	27.24

Means followed by the same letter do not differ by Tukey test at 5% significance. * Minimum Significant Difference

The interaction between cultivar and substrates also affected the DRM, and differences between the two cultivars were observed in substrates S6, S7, and S9. In the interaction with the two evaluated cultivars, the substrates S6 and S9 provided seedlings with higher DRM for the Simpson Black Seed cultivar than for Great Lakes cultivar. However, for the S7 substrate, the Great Lakes cultivar produced DRM seedlings superior to the Simpson

Black Seed cultivar (Table 4). The substrates that had sugarcane bagasse in their composition presented higher values of the dry mass of the root system than the substrates that did not have. Probably, this component provides better chemical and physical conditions for the good development of the root system of the seedlings. These differences in the dry mass of the root system resulted in more robust root systems that could allow greater absorption of water and nutrients. A good rooting of the seedlings and a rapid restart of the development of the plants are favored by tissues rich in dry matter after the stress caused by the transplanting process (Filgueira, 2005). The substrate used for the production of seedlings significantly influences the development of the root system, and this influence is mainly attributed to the quantity and size of the particles that define the aeration and the retention of necessary water for root growth (Kratz *et al*., 2013; Dutra *et al*., 2017; Andrino, 2018).

The different substrates influenced the NL per plant. A significant difference was observed for the two cultivars; however, no differences were observed for the interaction between the two studied factors. In addition to the NL per plant, the different substrates had effect on PH, FRM and DRM (Table 4).

Table 4. Number of leaves (NL), plant height (PH), dry root mass (DRM), and fresh root mass (FRM) as a function of different substrates.

Substrate	NL	PH (cm)	DRM (g)	FRM (g)
S1	3.750 e	4.500 e	0.130 e	0.858 ef
S2	7.250 a	14.013 abc	1.865 abc	1.671 bcd
S3	5.313 d	8.669 d	0.485 e	1.100 def
S4	6.750 ab	15.338 a	1.803 abc	1.260 def
S5	6.063 bcd	10.894 cd	1.049 d	2.284 ab
S6	6.875 ab	14.319 ab	2.133 a	2.323 ab
S7	6.063 bcd	11.663 bcd	1.453 cd	3.004 a
S8	6.938 ab	14.967 a	2.013 a	2.075 bc
S9	6.250 bc	14.764 ab	1.540 bcd	1.501 cde
S10	5.438 cd	14.344 ab	1.424 cd	0.743 f
MSD*	0.892	3.196	0.556	0.753
Average Overall	6.069	12.347	1.389	1.68
Default Error	0.192	0.688	0.120	0.16
CV (%)	8.95	15.76	24.34	27.24

Means followed by the same letter do not differ by Tukey test at 5% significance. * Minimum Significant Difference

The substrates S2, S4, S6 and S8, which contained commercial substrate in mixture with poultry or swine manure, with or without the addition of sugarcane bagasse, influenced the NL, being higher for the substrates with bovine waste than commercial substrate. A higher NL may stimulate the development of lettuce plants since the leaves are the main responsible for the production of assimilates in the plants. A greater production of assimilates may favor the growth and development of the plants before and after the transplanting process depending on the energy contribution, they contribute to the development of the root system (Table 4). The organic compound provided a higher NL compared to the substrates Plantmax® and washed sand (Medeiros *et al*., 2008). In general, the substratum constituted only by organic compound improved the length of the aerial part and root system and fresh mass of the aerial part and the root system, when compared with the commercial substratum (Monteiro *et al*., 2012).

For the PH and NL variables, the substrates S2, S4, S6 and S8 were superior to the commercial substrate and the commercial substrate mixed with bovine manure.

However, for PH, the substrates S9 and S10, as well as the substrates mentioned above, also favored the development of seedlings in height (Table 4). Medeiros *et al.* (2008) observed that the organic substrate provided higher shoot height than the commercial substrate and washed sand. Substrates containing only swine manure are not recommended for the production of lettuce seedlings (Medeiros *et al.,* 2016). Other authors observed that the commercial substrate presents better results than the substrates: sugar cane bagasse, filter cake, and the mixing in equal parts of cane bagasse with the commercial substrate (Freitas *et* al., 2013).

All treatments showed better results for the DMA, when using the commercial substrate combined with another constituent (S2 to S10), compared with the use of only commercial substrate (S1) (Table 2). Mixtures of substrate with pig manure, chicken bed, cane bagasse, chicken residue, bovine residue, (S2, S4, S6 and S8), presented the best results for PH (Table 4).

The substrates that contained commercial substrate and sugarcane bagasse in their composition, with or without the addition of wastes, favored the development of the root system, according to the FRM of the lettuce seedlings. In contrast, the seedlings with only commercial substrate (S1) and those cultivated with a substrate composed of the same mixture of swine, cattle, and poultry were the ones that provided the worse conditions for the development of the root system. Medeiros *et al.* (2008) observed a longer root length when lettuce seedlings were produced using organic substrate compared to a commercial substrate and washed sand as substrate. Freitas *et al.* (2013) observed that the commercial substratum Plantmax® only or in combination with any of the alternative substrates provided better results for the length parameter of the root system. Organic compounds as substrates in lettuce seedlings can replace a commercial substrate (Câmara, 2001).

CONCLUSIONS

The substrate with swine and poultry wastes was the one that favored the development of the aerial part of the seedlings and lettuce, while those with 33% of sugarcane bagasse favored the development of the root system. The commercial substrate, used in isolation, showed the lowest performance. Other proportions and mixtures can be tested in order to find new potential substrates for lettuce seedlings. To sum up, substrates with swine (for aerial part) and sugarcane bagasse (for root), had the best results.

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Revista Facultad Nacional de Agronomía

Effect of sowing density on the agronomic performance of Quinoa Nariño cultivar and the transmissivity of photosynthetically active radiation in the high tropics of Colombia

Efecto de la densidad de siembra sobre el desempeño agronómico de Quinua cultivar Nariño y la transmisividad de la radiación fotosintéticamente activa en el trópico alto de Colombia

https://doi.org/10.15446/rfnam.v74n2.90040

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Keywords:
Chenopodium quinoa
Crop yield
Dry matter
Phenology

ABSTRACT

Quinoa is a promissory crop in the Andean region, on average, grain yield was 1.62 t ha^{-1} with nearly 2,000 t in the year 2017. This study examined the response of quinoa to the radiation transmission, growth, and development of the crop in different stages, under three planting densities in order to determine the differential responses and identify which aspects are determinants in the planting and development process and crop yield. For the present experiment, quinoa was sowed in three different densities: D1 65,500, D2 83,333, and D3 156,250 plants per ha. This study measured the percentage of canopy PAR transmission, distribution of matter on root, stem, leaf, and panicle, leaf development in leaf area and leaf area index, yield components, weight of 1,000 grains, and harvest index. The results showed that sowing density had no impact on PAR transmission, lower sowing densities obtained the best dry weight of panicle at the end of the production cycle, better yields, and best grain weight. To conclude, the sowing density affects different yield components, while all of them allow the plant to generate the best response within the production cycle.

Palabras clave:
Chenopodium quinoa
Rendimiento
Biomasa
Fenología

RESUMEN

La quinua es un cultivo promisorio en la región Andina, en promedio, el rendimiento de grano fue de 1,62 t ha^{-1}, con cerca de 2.000 t en el año 2017. El presente estudio evaluó la respuesta de la quinua a la transmisión de la radiación, el crecimiento y desarrollo del cultivo en diferentes etapas, bajo tres densidades de siembra, con el fin de buscar respuestas diferenciales e identificar cuáles aspectos son determinantes en el desarrollo de la planta, así como, para el rendimiento del cultivo. Para responder al objetivo, la quinua fue sembrada en tres densidades: D1 65.500, D2 83.333 y D3 156.250 plantas por ha. Este estudio midió el porcentaje de transmisión PAR del dosel, distribución de materia en raíz, tallo, hoja y panícula, el área foliar e índice de área foliar, componentes de rendimiento, peso de 1.000 granos e índice de cosecha. Se encontró que la densidad de siembra no tuvo efecto en la transmisión de PAR, bajas densidades de siembra presentaron el mejor peso seco de la panícula en el ciclo de producción final, mejores rendimientos y el mejor peso del grano. Se concluyó que, la densidad de siembra afecta los diferentes componentes del rendimiento y la suma de ellos permite que la planta obtenga la mejor respuesta en el ciclo de producción.

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Received: January 01, 2021; Accepted: February 25, 2021
Rev. Fac. Nac. Agron. Medellín 74(2): 9491-9497. 2021

ISSN 0304-2847 / e-ISSN 2248-7026

Quinoa (*Chenopodium quinoa* Wild) is a crop originated in the South American Andean mountain chain (Tapia *et al*., 1979), with a diversity of ecotypes adapted to different environments (De Santis *et al*., 2018). The grains of quinoa contain protein of outstanding quality. They are better balanced in terms of amino acid composition than most other cereals (Tapia, 2000). Also, it has great agronomic potential for tropical and temperate conditions. Currently, the interest in this crop has been growing around the world (Asher *et al*., 2020). Quinoa crop has received a great attention in continents such as Europe, North America, Asia and Africa, because of its ability to be productive under various environmental stresses (Bazile *et al*., 2016; Gesinski, 2008; Gomez-Pando, 2015).

Quinoa is used as a traditional cereal but without being part of the grass family. It belongs to the dicotyledonous family Amarantaceae. It is an herbaceous plant, 3 m high in some varieties, with high phenotypic plasticity (Becker *et al.,* 2017). Morphology, coloration and phenology depend on ecotype in agroecological zones where they are cultivated (Apaza *et al*., 2013). The Nariño cv is originated from selections that have been adapted to the conditions of the department of Nariño in southern Colombia. It is a quinoa with an amaranthiform panicle, which is characteristic of Real Quinua, a name given by the large light-colored grain size, that presents good protein content (Veloza *et al*., 2016). Temperature and photoperiod have been widely studied in terms of their impact on growth and development. In contrast, hydric status and solar radiation have scarce researches (Razzaghi *et al*., 2012; Ruiz and Bertero, 2008). On the other hand, other studies have found that temperature and salinity could influence development and yield. Nonetheless, the plant could diminish these effects due to its great phenotypic plasticity (Becker *et al*., 2017).

Increasing sowing density is one of the main practices used to improve solar radiation capture by crops (Idinoba *et al*., 2002). The accumulation of biomass only depends on the incident photosynthetically active radiation (PAR) in absence of stress conditions. The incident PAR varies according to latitude, season, date of sowing and phenology of plants. The relation between incident PAR and the biomass increases is denominated as radiation use efficiency (RUE) (Monteith and Moss, 1977). This expresses the relative mass accumulation of the crop to the amount of light intercepted by the leaves. The arrangement of the crop rows can alter the light distribution, leading changes in the intercepted PAR. Also, changes in sunlight distribution cause variations in crop yield (Liu *et al*., 2017).

Ruiz and Bertero (2008) in a study conducted in Quinoa with stable conditions of nutrient and availability of southern Chile found that the RUE is directly affected by the leaf area index (LAI) through modifying the distribution of radiation inside the plant. Besides, improvements in the period where PAR interception was below 50% is key to increasing the biomass gain. Alternatives to modify LAI are the uses of varieties with a different leaf arrangement or a modification of the sowing density (Liu *et al*., 2012). Different studies of sowing density of quinoa have been carried out. These have indicated that plot arrangements of 80 to 327 plants per m^2 (near 1 million plants ha^{-1}) with a space between rows (50 cm) are optimum in order to obtain the best yield in temperate conditions in Denmark (Jacobsen *et al*., 1994). In contrast to the results found, in temperate conditions in Brazil, 100,000 plants ha^{-1} is one of the best sowing densities for quinoa (Spehar and Rocha, 2009).

The aim of this study was to evaluate the response of the quinoa crop Nariño cv to the radiation interception, growth, development and yield, taking into account three different planting densities in high tropical conditions in Colombia.

MATERIALS AND METHODS

Experimental design and growing conditions

The research was developed at the Universidad de Ciencias Aplicadas y Ambientales U.D.C.A, at the countryside area 'El Remanso' located in the north of Bogota, Colombia (4°47'57.98"N74°2'47.17"W, 2,560 masl). The experiment was performed in the second semester of 2016. Plant material was Quinoa Nariño cv (Veloza *et al*., 2016). A completely randomized experimental design was applied with three treatments (sowing densities), three repetitions, and the experimental unit was three plants. Sowing densities were D1 65,500 plants ha^{-1} (0.2x0.8 m); D2 83,333 plants ha^{-1} (0.15x0.8 m); D3 156,250 plants ha^{-1} (0.08x0.8 m). Planting was

developed in loamy soil with pH 6.9 and 14% of organic matter. The seed was disposed at 3 cm soil depth, drip irrigation was provided in the germination and vegetative phase.

Response variables

Canopy transmission of PAR radiation (percentage) was measured in four phenological stages: vegetative, panicle development, flowering, and milky grain. Measurements were made using a lineal ceptometer model AccuPAR LP-80. Transmission percentage was determined by using this equation: TR(%)=(TRx100)/IR, Incident Radiation (IR) as 100% of total energy and Transmitted Radiation (TR) as the transmitted energy through canopy. This Equation is related to energy balance in leaf and plant canopy (Lambers *et al*., 2008). Three plants were sampled by each repetition, 3 repetition by each sowing density. Rows were north to south, and quantum bar were placed east to west.

IR was determined by the ceptometer in total solar exposition, with an external PAR sensor included in the ceptometer and TR between lower canopy position and soil with the quantum bar of the ceptometer. Data were obtained every day between 12:00 and 13:00 hours.

Leaf area was determined by destructive measurements in four phenological stages: vegetative (46 days after sowing, DAS), branch development (76 DAS), panicle development (106 DAS), and flowering (134 DAS). For each plant, data were measured with CL-202 portable laser area meter from CID Bio Science Inc.

Dry weight (DW) measurements were made for root, stem, leaf, and panicle according to Hunt (1978) protocol. The samples were put in an oven with temperatures up to 80 °C and measured every 24 h, until they obtained a constant weight.

Three plants per repetition were sampled to determine yield, total grain weight per plant, total plant weight, the weight of 1,000 grains, and harvest index (HI). HI was determined by the equation: HI=Grain Yield per plant/total fresh plant weight Yield (t ha^{-1}) was determined by a thresher, harvesting four lineal meters of the central area per repetition.

Statistical analysis

Data were subjected to analysis of variance (ANOVA). Mean comparison was performed using the Tukey honestly significant difference (HSD). Grains and plant weight data showed heteroscedasticity, and the data was transformed with a natural logarithm. Analyses and calculations were developed using IBM SPSS version 23.

RESULTS AND DISCUSSION

The values of plant density (D1, D2, D3) did not have a significant effect on the transmission percentage of solar radiation in any of the phenological stages (Figure 1).

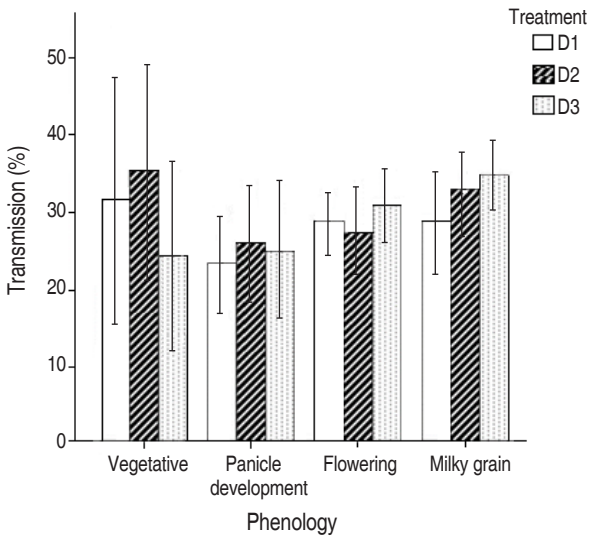

Figure 1. Transmission percentage of photosynthetically active radiation (PAR) of Quinoa canopy. Phenology stages vegetative (65 DAS), panicle development (108 DAS), flowering (124 DAS), milky grain (137 DAS). No statistically significant differences were obtained in all measurements (P>0.05), according to Tukey test. Vertical lines correspond to standard deviation.

PAR percentage transmission in all phenological stages was below 40% of transmission. The highest value was obtained in shoot growth by D2 with 34.49%, with a theoretical interception of 65.06% of PAR values upper 80% Ruiz *et al.,* (2008).

Increasing sowing density did not show effects; this is the reason why PAR transmission was not statistically significant in all treatments and phenology stages. Leaf area index is related to this result, since the values were similar in all densities, and leaf area is related with interceptibity of radiation, affecting also transmissivity as another component of energy balance as Bosco *et al*. (2020) showed in their study using apple orchards. In root DW, samples showed a significant difference only at 76 DAS (Figure 2A). Treatment D1 obtained the higher root DW values, followed by D3, with no statistical differences between them. In the case of stem dry weight (Figure 2B), measurements were taken at 76, 106, and 137 showing no significant differences. After that, at 46 DAS plants sowed at D1 density, showed the best stem DW. For leaf DW (Figure 2C), statistical differences were found at 106 DAS. The plant density D1 obtained the highest DW, followed by D2 and D3, with significant differences between them. Besides, D1 showed the highest leaf DW at 137 DAS, followed by D2 and D3, with statistical differences between D2 and D3.

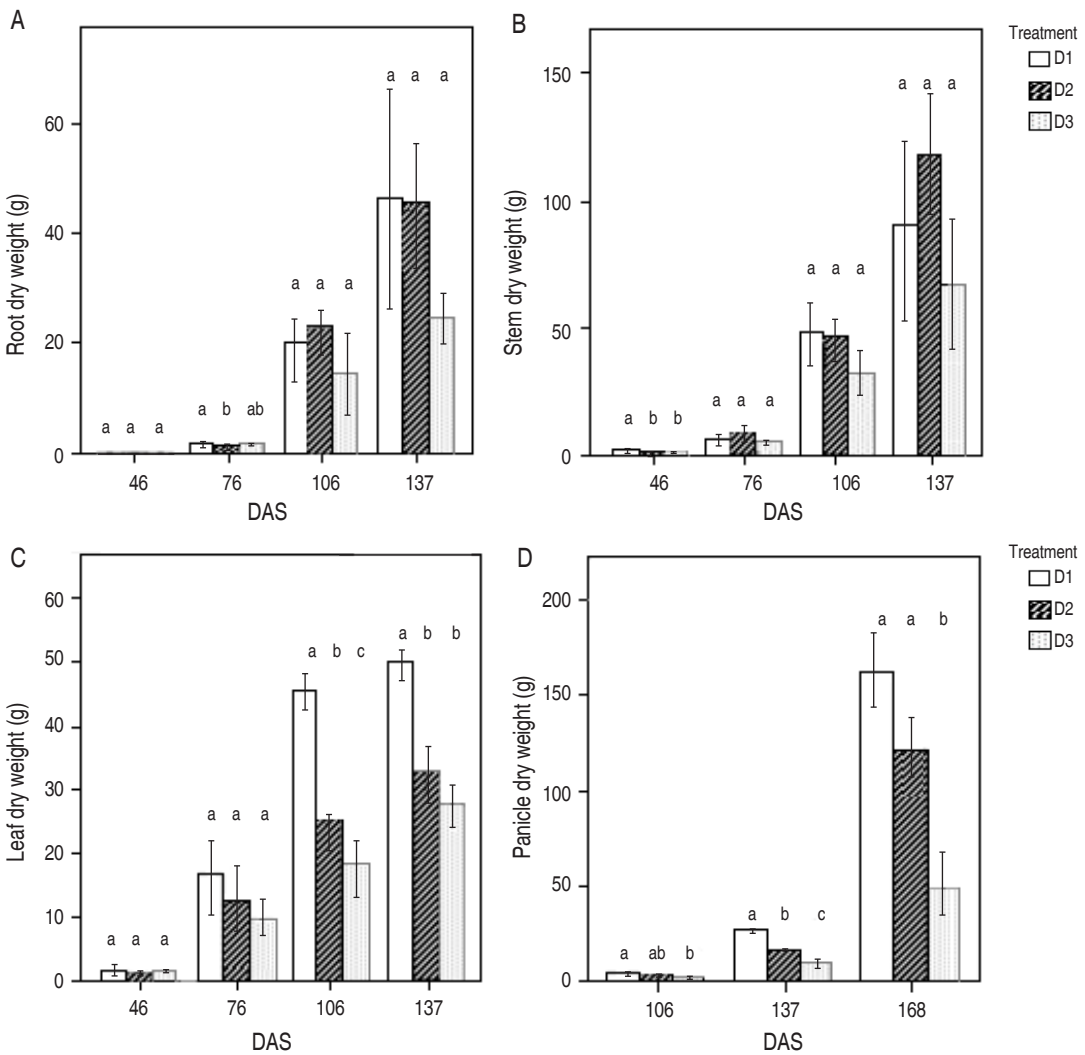

Figure 2. Mean values of dry weight per plant section. A, B, C: measurements were taken at 46, 76, 106, and 137 DAS; D: panicle DW measurements were taken at 106, 137, and 168 DAS. The results with the different letters in each phenological phase were statistically significant ($p<0.05$) and probed the Tukey test. Vertical lines correspond to standard deviation.

According to Figure 2D, plants sowed at D1 density showed a higher significant panicle DW gain in all measurements (106, 137, and 168 DAS). In contrast, D3 registered the lowest values of DW in all measurements. In quinoa root DW, Tarek *et al*. (2017) found in samples taken at 75 DAS that plants growing under stress conditions generate more DW because they need to grow larger to reach the water. At 75, D1 was the treatment with less plant density, and the shoot (stem and leaf) was not thoroughly developed. This allowed a treatment more susceptible to water loss due to higher crop evapotranspiration (Moradi *et al*., 2011). However, once the plants were under a complete leaf area, the differences vanished. Researchers have identified that above-ground DW could get a unique response to an increase of salinity in the soil; otherwise, factors like temperature did not affect DW (Becker *et al*., 2017).

Significant leaf area between sowing densities were obtained after 137 DAS, where D2 obtained the highest one (Figure 3A). For the LAI at 46 and 76 DAS, significant results were obtained: in both measurements, the highest values according to plant density were D3, D2, and D1. Nonetheless, there were no significant differences among them (Figure 3B). The present study showed that the effect of sowing density on leaf area is only observed at the end of the production cycle. This behavior is because of the adaptability of quinoa to all kinds of environments (Jacobsen *et al*., 2003); therefore, the decrease of leaf area is possible under drought conditions. Fghire *et al*. (2015) found LAI values higher than 4 in well-irrigation conditions in Morocco.

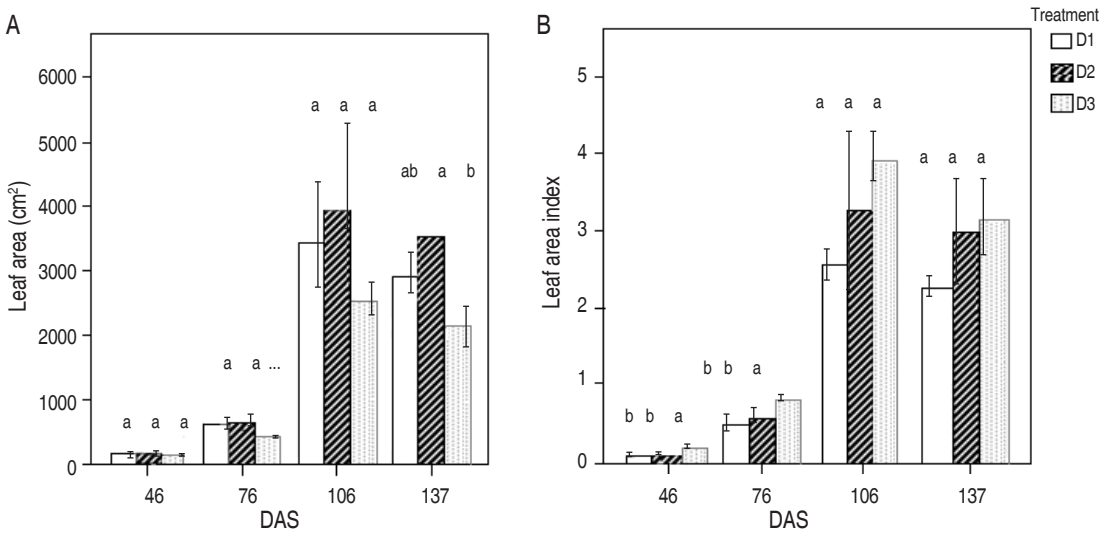

Figure 3. Mean values per plant of leaf area (A) and leaf area index (B). Measurements were taken at 46, 76, 106, and 137 (DAS). Values with different letters were statistically significant according to the Tukey HSD test ($P<0.05$). Verticals lines correspond to standard deviation.

Concerning plant densities, the D1 arrangement was the best density in terms of yield (Table 1), followed by D2. The lowest grain production was obtained by plants sowed at D3 density. Significant differences in the weight of 1,000 grains were obtained by D1 treatment. D3 had the lowest values for this variable. Moreover, for the harvest index, significant differences among densities were not found.

The optimum density of plants in the present experiment was D1 with 5.28 t ha^{-1}, compared to the reports of Blanca Nariño (Colombia) Hualhuas and Mantaro cultivars with yields around 3.5-4.5 t ha^{-1} (Zurita-Silva *et al*., 2014). A Peruvian genotype (4B-216) was reported with a yield of 4.168 t ha^{-1} (Garrido *et al*., 2013).

Similar HI values (36.5%) were found by Hussain *et al*. (2018) in Dubai with control samples of Chilean and USA varieties. For this study, HI values were similar in yield while they were higher in control conditions. Spehar and Rocha (2009) identified that the increase in planting density is not correlated with an increase in yield. An evaluation of nine varieties of quinoa showed that yield and harvest have a high correlation with environment and genotype (Garrido *et al*., 2013).

Table 1. Yield weight of 1,000 grains, and plant weight and harvest index (HI) for different planting densities of Quinoa.

Treatment	Yield (t ha^{-1})	Weight of 1,000 grains (g)	HI (%)
D1	5.28 a	3.5586 a	41.5 a
D2	4.41 ab	3.3316 ab	38.6 a
D3	3.26 b	3.2923 b	34.8 a
Significance	**	**	NS

**Significant effect at 0.05; NS: no significance. Values within columns followed by different letters were statistically significant to the Tukey HSD test (P<0.05).

The present study identified that sowing density (5 plants m^{-1}) could affect yield while HI does not. Regarding the weight of 1,000 grains, similar responses were found in a mutant quinoa plant (3.5 g) in a study performed in Peru (Pando *et al*., 2017). In a study carried out in Nariño, Colombia, the best response over this variable was obtained by Piartal cultivar with 3.45 g (Delgado *et al*., 2009). Weight of grains and the number of plants per ha were the components responsible for increasing the yield. Plants with less sow density allowed developing grains with the best weight as well as more grains per plant, allowing to increase yield and HI (Jia *et al*., 2018 Similar results were obtained by Eisa *et al*. (2018), who reported that increasing plant led a significant decrease in the weight of 1,000-grains, using similar densities to this research (4 and 15 plants per LM). Whereas Erazzú *et al*. (2016) found that the increase plant sowing density (27 plants per LM), led to a decrease grain yield.

CONCLUSION

No differences were found in the percentage of radiation transmission for different planting densities through the development of quinoa phenological stages. However, there was a significant accumulation of dry matter in leaf and panicle at the final phenological phases related to the differences in leaf area in the milky grain phase. This probably means that more leaves are translocating photoassimilates to a higher number of grains, reflected in larger panicles, since a higher content of dry matter was evidenced in the D1 treatment. Successively, the lower density presented a higher yield for the variety evaluated, which was characterized by presenting a panicle of amarantiform shape, with larger panicles and grains similar to Real quinoa varieties, compared to varieties of glomerulates panicles not evaluated in this research. For future studies, it is convenient to extend the evaluation to varieties of quinoa of different panicle shapes in order to evaluate the best planting densities obtained in this research.

ACKNOWLEDGMENTS

We thank to the Universidad de Ciencias Aplicadas y Ambientales (U.D.C.A) for the economic support to perform the research.

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Review article

https://revistas.unal.edu.co/index.php/refame

Revista Facultad Nacional de Agronomía

Cadmium in soil and cacao beans of Peruvian and South American origin

UNIVERSIDAD NACIONAL DE COLOMBIA

Cadmio en suelos y granos de cacao de origen peruano y sudamericano

https://doi.org/10.15446/rfnam.v74n2.91107

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ABSTRACT

Cadmium tends to bioaccumulate in different parts of cacao plant and its consumption can lead to serious health complications; due to this, the European Union (EU) established limits for tolerable concentrations of cadmium in cacao products as a preventive measure, which took effect as of January 2019. In South America and Peru, a sustained growth in cacao production has been recorded over the last 10 years, but scientific studies reveal that in some areas the cadmium levels of the soil and cacao beans exceed those established by the EU, thus, jeopardizing marketing and export possibilities to the EU. With this in mind, the purpose of this review was to compile information on the cadmium that is available in the soil, its accumulation in cacao beans, and the advances in treatment technologies; as well as to analyze the potential effects this has on cacao exports of South American origin, using Peru as a case analysis.

Keywords:
Bioaccumulation
Cadmium levels
European Union regulations
Geo-distribution
Treatment technologies

RESUMEN

El cadmio tiende a bioacumularse en distintas partes de la planta de cacao y su consumo puede conducir a graves complicaciones de salud; por ello, como medida preventiva, la Unión Europea (UE) estableció concentraciones tolerables de cadmio a productos derivados del cacao, el cual entró en vigencia desde enero del 2019. En Sudamérica y el Perú la producción de cacao registra un crecimiento sostenido en los últimos 10 años y estudios científicos revelan que algunas zonas presentan niveles de cadmio en suelos y granos que superan lo establecido por la UE, poniendo en riesgo sus posibilidades de comercialización y exportación hacia la UE. En ese sentido, el propósito de esta revisión fue compilar información sobre el cadmio disponible en suelos y su acumulación en granos de cacao, los avances en tecnologías de tratamiento y analizar los potenciales efectos en las exportaciones del cacao de origen sudamericano, tomando Perú como análisis de caso.

Palabras clave:
Bioacumulación
Niveles de cadmio
Reglamento de la Union Europea
Geo distribución
Tecnologías de tratamiento

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Received: January 26, 2021; Accepted: February 24, 2021
Rev. Fac. Nac. Agron. Medellín 74(2): 9499-9515. 2021

ISSN 0304-2847 / e-ISSN 2248-7026

INTRODUCTION

Cadmium is a heavy metal with no known biological function, the physicochemical properties of which are between Zn and Hg (Antoine *et al*., 2017). It was discovered in Germany in 1817 by Friedrich Stromeyer as an impurity in zinc carbonate ($ZnCO_3$) (Pérez and Azcona 2012); its various applications in the industry started 50 years ago (Pérez and Azcona, 2012; Gunnar, 2013). However, it is currently causing a number of complications in vital organs such as lungs, kidneys, liver and bones either by inalation or ingestion, becoming in a potential threat to human health (Reyes *et al*., 2016; Ali *et al*., 2020). Despite being in low concentrations when compared to other metals, the impact on health, due to its high mobility and bioaccumulative power is alarming (Reyes *et al*., 2016; Engbersen *et al*., 2019). Even at trace levels cadmium can cause serious health complications (Maddela *et al*. 2020).

There was an incident in Japan in the 1950s where the people living on the banks of the Jintsu River were affected by the consumption of rice from crops that were contaminated with cadmium from mining (Reyes *et al*., 2016; Hernández-Baranda *et al*., 2019). From there, a series of studies was initiated which lead to cadmium, as well as lead, mercury, and chromium, to be considered as the elements most dangerous to human health (Casteblanco, 2018; Engbersen *et al*., 2019); particularly, because of their cumulative nature which can cause a series of damages to one's health (Prieto *et al*., 2009; Reyes *et al*., 2016; Maddela *et al*., 2020).

Soil generally has a low cadmium content (Kabata-Pendias, 2010), but regardless of the level and origin, the dynamics of the metal depend on its chemical form and the characteristics of the soil (Bravo *et al*., 2014; Díaz *et al*., 2018; Scaccabarozzi *et al*., 2020). This limits or contributes to the mobilization and uptake of cadmium from cacao plants (Gramlich *et al*. 2017; Zug *et al*. 2019); in some plant structures it is accumulative (Hernández-Baranda *et al*., 2017; Tantalean and Huauya, 2017; Casteblanco, 2018), and reaches concentrations that are higher than in the soil itself (Chávez *et al*., 2015; Díaz *et al*., 2018; Oliva *et al*., 2020). Due to this, the EU and some other countries have implemented standards to classify agricultural soil. Also, the EU has established tolerable limits for cacao beans and its by-products that are imported.

Taking into account the studies that warn of high levels of cadmium in cacao beans and processed cacao products of South American origin (Chávez *et al*., 2015; Lanza *et al*., 2016; Arévalo-Gardini *et al*., 2017; Gramlich *et al*., 2017; Díaz *et al*., 2018; Argüello *et al*., 2019), the recent EU provision, Regulation No. 488/2014, which entered into effect in 2019, sets tolerable values to be between 0.1 and 0.80 µg g^{-1} for products derived from cacao (EU, 2014; Jiménez, 2015). This will put the quality and export possibilities of cacao from Latin America at risk, and in particular those coming from Peru, whose main market is the EU, which makes up 76% of their exports (MINAGRI, 2019).

Therefore, the objective of this document was to evaluate, through the interpolation of data, the presence of cadmium available in the soil, its bioaccumulation in cocoa beans, and the technological advances for controlling it; as well as to analyze the potential effects on Latin America exports volumes, using Peru as a case analysis.

Cadmium in the soil

The U.S. Environmental Protection Agency (USEPA) established 0.43 µg g^{-1} to be a critical level of total cadmium in agricultural soil (USEPA, 2002). On the other hand, the EU through the Kelley Directive, indicated that the typical values in uncontaminated soil with cadmium are between 0 and 1 µg g^{-1} (Acevedo *et al*., 2005) and the recent supreme decree issued by the Peruvian Ministry of Environment, DS N° 011-2017 (MINAM, 2017) approved environmental quality standards (EQS) for soil, setting 1.4 µg g^{-1} as the maximum limit for the cadmium values of agricultural soil.

The natural average level of cadmium in agricultural soil fluctuates between 0.01 to 7 µg g^{-1} (Bohn *et al*., 1993); although more specific studies have found it to be between 0.07 and 1.1 µg g^{-1}, with a natural base level of 0.5 µg g^{-1} (Kabata-Pendias, 2010). In addition, rock composition can elevate the cadmium levels in soil (Argüello *et al*., 2019); generally following the natural order of their evolution, with the lowest values being found in the most evolved soils, which have an acidic pH, low cation exchange capacity values and thick textures (Pérez and Azcona, 2012). Also, alluvial soil has been shown to have higher levels of available cadmium

when compared with residual cadmium (Tantalean and Huauya, 2017; Scaccabarozzi *et al*., 2020); and the contribution of natural processes to the cadmium contamination of the soil is three to ten times less than in anthropogenic sources (He *et al*., 2015).

Naturally, soils have varing levels of cadmium, and the availability is subject to their physicochemical and biological properties; studies reveal relationships between cadmium and pH (Arévalo-Gardini *et al*., 2016; Florida *et al*., 2018). The higher the pH level of the soil, the greater the cadmium retention and the lower the cadmium contamination in cacao beans, according to the Kelley Directive (Acevedo *et al*., 2005). In addition, the cadmium concentration and mobility are influenced by the percentage of clay, the presence and type of organic matter (Bravo *et al*., 2014), the available cadmium (Sánchez *et al*., 2011; Gramlich *et al*., 2018), the cation exchange capacity, and the amount of mangnesium and zinc (Degryse *et al*., 2009; He *et al*., 2015; Arévalo-Hernández *et al*., 2017; Argüello *et al*., 2019; Zug *et al*., 2019). These authors suggest that the aforementioned indicators have a direct effect on the plant's absorption.

Geo-distribution of available cadmium soil

There are reports of high levels of cadmium in the soil of Latin America. Table 1 shows the average of the sampling work that is carried out at regional levels and even at national levels in some countries. The results reveal that on average, South America has 0.42 µg g^{-1}, being categorized as having soil that is not contaminated by cadmium, according to the USEPA (0.43 µg g^{-1}), which has the most stringent regulations established so far for agricultural soil.

Table 1. Available cadmium in the soil of some cacao-producing countries of South America.

References	Country	Samples	Cd Level (µg g^{-1})
Araujo-Abad *et al.* (2020)	Ecuador	--	0.304
Argüello *et al.* (2019)	Ecuador	560	0.44
Barraza *et al.* (2019)	Ecuador	145	0.20
Barraza *et al.* (2017)	Ecuador	113	0.44
Chávez *et al.* (2015)	Ecuador	76	0.85
Mite *et al.* (2010)	Ecuador	568	0.49
Chambi (2010)	Bolivia	18	0.39
Gramlich *et al.* (2017)	Bolivia	--	0.20
Aguirre-Forero *et al.* (2021)	Colombia	--	0.02
Silva (2019)	Colombia	--	0.096
Charrupi and Martínez (2017)	Colombia	--	1.15
Marrugo-Negrete *et al.* (2017)	Colombia	--	0.04
Almeida (2016)	Brasil	50	0.705
*	Peru	160	0.33
Total Samples		1727	
Average			0.40

* Calculated by the author (Table 2). -- Not specified.

Peru is considered megadiverse country due to its bioclimates and the fact that is possesses coastal, mountain and tropical soil (Pulgar, 2014). In addition, it is the center of the origin of the greatest diversity of cacao in the world (Motamayor *et al*., 2008); thus, it is a good representation of a caco producing country in South America for the case analysis. Table 2 shows Peruvian scientific reports from the different districts, provinces and regions of the country.

Table 2. Available cadmium in the soil of different cacao producing areas within Peru.

Zone	Region	Province	References	Cd Level (µg g^{-1})	UTM Coordinates
North	Tumbes	Tumbes	4	0.50	562379E-9606058N
			4	0.26	556998E-9604790N
	Piura	Piura	4	0.48	538295E-9427857N
		Huancabamba	4	0.14	672373E-9422057N
		Morropón	4	0.53	592923E-9436422N
		San Ignacio	4	0.01	720769E-9431431N
	Amazonas	Bagua	4	0.11	773547E-9376440N
		Bagua	7	1.46	--
		Condorcanqui	4	0.01	783829E-9364136N
	San Martin	San Martin	1	0.27	354517E-9278816N
		Bellavista	4	0.20	324522E-9219718N
			1	0.32	326533E-9215295N
		El Dorado	1	0.27	313538E-9268407N
		Lamas	1	0.22	332615E-9290811N
		Huallaga	4	0.29	304650E-9231329N
		Mariscal Cáceres	1	0.28	312023E-9205915N
			4	0.21	300970E-9192099N
		Moyobamba	1	0.16	286619E-9329588N
		Picota	1	0.33	352976E-9235603N
		Rioja	1	0.22	261893E-9329417N
		Tocache	1	0.23	332630E-9092486N
		Bambamarca-Tocache	5	0.24	647657E-9097795N
		Tananta-Tocache	5	0.16	326325E-9102289N
		Nvo. Progreso-Tocache	5	0.19	354326E-9065466N
Center	Junín	Satipo	4	0.10	539354E-8755739N
	Huánuco	Rupa Rupa	6	0.53	394588E-8978855N
			3	0.63	391952E-8966760N
			5	0.45	395116E-8982497N
		Supte San Jorge	5	0.54	393951E-8971537N
		Pueblo Nuevo	3	0.41	383134E-8994796N
		Puerto Ángel	5	0.18	382690E-8996783N
		Castillo Grande	3	0.29	386765E-8978110N
			3	0.82	388995E-8976876N
		J. Crespo y Castillo	3	0.41	383134E-8994796N
			3	0.16	389956E-8988252N
		San José Pucate	5	0.26	374175E-9014524N
		Marona	3	0.26	396097E-8979819N
		Dámaso Beraun	3	0.40	395359E-8959585N
		Aalto Sanjuan	5	0.30	411020E-8961339N
		Bella-Monzón	5	0.29	385888E-8969250N
		Paraíso	5	0.32	347572E-9059513N
		Venenillo	5	0.22	379875E-8993949N
	Ucayali	Callería	5	0.37	546513E-9071823N
		Irazola	5	0.86	444682E-9001025N
		A. Bon Humbolt	5	0.29	498145E-9027812N
		Padre Abad	2	0.23	445791E-9015463N
		Nuevo Horizonte	5	0.28	470854E-9022059N
		Nuevo Tahuantinsuyo	5	0.26	459718E-9013764N
South	Cusco	La Convención	4	0.01	747811E-8577789N
Average				0.33±0.18	

-- Not specified. Data of four regions (Madre de Dios, Ucayali, San Martín and Amazonas) were not considered in the geo-distribution.
Source: 1 GRSM (2019); 2 Florida *et al.* (2019); 3 Florida *et al.* (2018); 4 Arévalo-Hernández *et al.* (2017); 5 COPAIN (2014); 6 Huamani *et al.* (2012); 7 Oliva *et al.* (2020).

Using these data of averages, a spatial analysis was performed through interpolation with the inverse distance weighting (IDW) extension in ArcGIS, version ArcMap10.5, to determine the geo-distribution of the available cadmium in the terirritory of Peru (Figure 2).

Figure 1, through interpolation, shows the geo-distribution of the levels of available cadmium in soil and that it is determined that 100% of the territory is below the tolerable limit for agricultural soil, as set by Peruvian regulations and only 1.11% approach, but do not exceed EU limits (Kelley Directive); therefore, according to the interpolation, Peru has soil which is intended for cacao production and is not contaminated by cadmium.

Cadmium in cacao beans

The EU with Regulation No. 488/2014, established tolerable limits on cacao and chocolate derivatives: for milk chocolate with cacao solids less than 30%, 0.1 µg g^{-1}; for chocolate with cacao solids of less than 50% and milk chocolates with cacao solids greater than or equal to 30%, 0.3 µg g^{-1}; for chocolates with cacao solids greater than or equal to 50%, 0.8 µg g^{-1}; and for cocoa powder, 0.6 µg g^{-1} (EU, 2014).

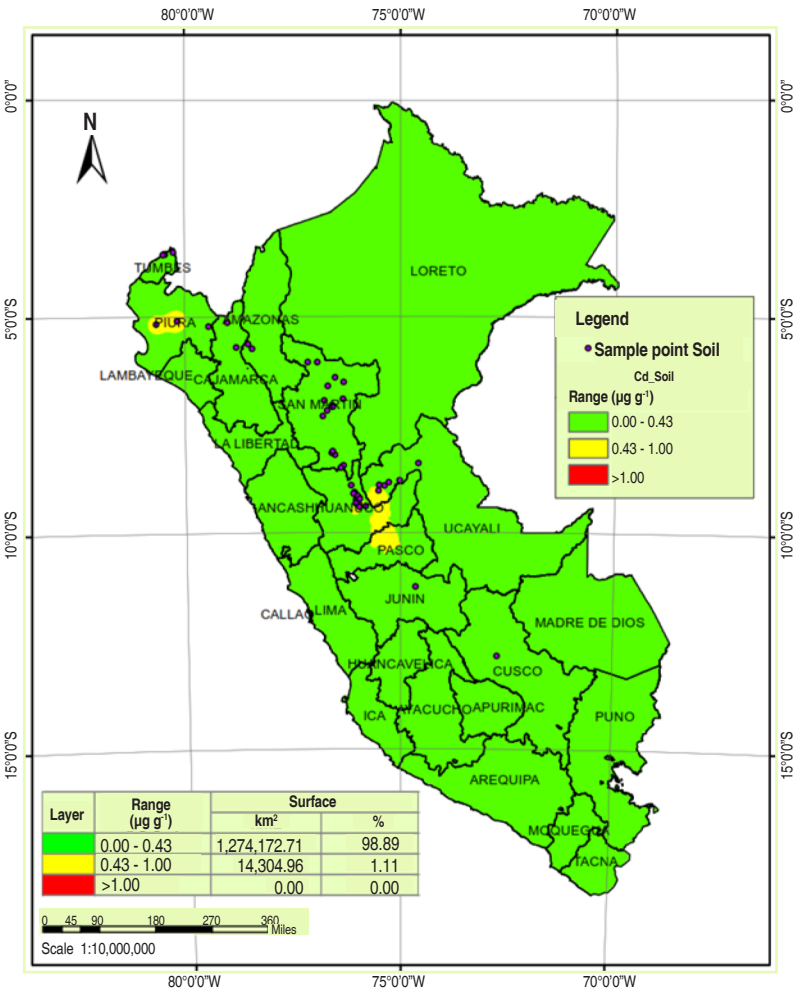

Figure 1. Geo-distribution of available cadmium in the soil of the Peruvian territory

According to some countries, such as the United States, Food and Drug Administration (FDA) gives specifications for cadmium levels in bottled water, up to 0.005 mg L^{-1} according to the Code of Federal Regulations, 2013, Sec. 165.110. Title 21 of the Federal Code for Food and Medicines, chapter 163, details the different specifications for cacao products; there are no established levels for pollutants in this document, specifically for cadmium. In

the case of Peru, there is no specific standard for cadmium levels in cacao, which is similar to other countries in the region, such as Chile (Food Health Regulations, title IV referring to pollutants), Bolivia (National Directorate of Standardization), Ecuador (NTE INEN 621: 2010) and Venezuela (La COVENIN 50: 1995 standard). It is important to note that the United States, Russia, Canada and Japan do not have limits for tolerable amounts of cadmium in cacao derivatives (Jiménez, 2015); thus, these could be alternative destinations for exports that exceed EU limits.

In this context, the soil has a low cadmium content, referring to both Peru (Figure 1), and the reports available for South American (Table 1), but research warns that cacao beans can capture cadmium and reach concentrations that are higher than the soil itself (Chávez *et al*., 2015; Díaz *et al*., 2018; Engbersen *et al*., 2019; Oliva *et al*., 2020). Moreover, most agree that bioaccumulation is higher in roots, leaves, pods and beans, with or without shell (Ramtahal *et al*., 2019; Barraza *et al*., 2017; Gramlich *et al*., 2017; Oliva *et al*., 2020), which is an aspect that can be analyzed.

Geo-distribution of cadmium in beans

Table 3 shows the average of the work that was done at regional levels in South American countries. The results reveal that, on average, the cadmium levels of cacao exceed those established by EU Regulation No. 488/2014 (maximum 0.8 µg g^{-1} cadmium). The results also show that beans have 2.1 times more cadmium in relation to the cadmium available in soil. This shows the bioaccumulation capacity of cacao and the potential impact on the possibilities of marketing this product to the EU, the main export market for cacao produced in Latin America.

Table 3. Total cadmium in cacao beans from some cacao-producing countries of South America.

References	Country	Samples	Cadmium levels (µg g^{-1})
Argüello *et al*. (2019)	Ecuador	560	0.9
Barraza *et al*. (2019)	Ecuador	145	0.4
Barraza *et al*. (2017)	Ecuador	113	1.12
Chávez *et al*. (2015)	Ecuador	76	0.94
Mite *et al*. (2010)	Ecuador	803	0.84
Romero-Estévez et al. (2019)	Ecuador	--	0.75
Lanza *et al*. (2016)	Venezuela	NE	1.62
Oliveira *et al*. (2019)	Brasil	61	0.129
Aguirre-Forero *et al*. (2021)	Colombia	NE	0.66
Niño (2015)	Colombia	NE	1.62
Gramlich et al. (2017)	Bolivia	NE	0.21
*	Perú	160	0.91
Total samples		1973	
Average			0.84

* Calculated by the author.
-- Not specified

Table 4 shows the results of research throughout Peru and among different cacao genotypes; data was statistically analyzed with the Stata program (R) 15.1, to create box diagrams (Figures 2A), using the Minitab * 18.1 program, in order to determine interquartile ranges (Figure 2B).

Figure 2A, shows that the average concentration of cadmium in Peruvian soil used for cacao production is 0.29±0.18 µg g^{-1} and in the beans it is 0.91±0.48 µg g^{-1}, this latter is 3.13 times higher than the value calculated for South America (2.1 times more cadmium in relation to the available cadmium in the soil). Also, when looking at the interquartile range (Figure 2B), 50% of the cadmium in the soil is between 0.02 and 0.23, while in the beans it is between 0.18 and 1.52 µg g^{-1}.

Table 4. Total cadmium in cacao beans from different areas of production in Peru.

Zone	Region	Province	References	Cd levels (µg g^{-1})	UTM Coordinates
North	Tumbes	Tumbes	4	1.78	559145E- 9608347N
	Piura	Piura	4	1.55	558018E- 9608353N
		Jaén	4	0.75	743205E- 9379500N
	Amazonas	Bagua	4	0.80	746080E- 9370593N
		Imaza	3	0.41	778456E- 9444312N
	San Martin	San Martin	4	0.78	791650E- 9278816N
		Santa Cruz-Tocache	5	1.41	354517E- 9054092N
		Shapaja-Tocache	5	1.11	358296E- 9082526N
		Tananta-Tocache	5	0.93	350341E- 9102541N
		Bambamarca	5	0.96	326715E- 9103226N
Center	Junín	Satipo	4	0.45	322870E- 8766373N
	Huánuco	Rupa Rupa	2	1.42	533950E- 8966760N
			2	1.93	391952E- 8982497N
		Bella	5	1.01	395116E- 8969187N
		Supte San Jorge	2	0.30	386028E- 8973837N
		Marona	1	0.27	403663E- 8979819N
		Marona Baja	5	1.00	396097E- 8978419N
		Pueblo Nuevo	2	1.37	396680E- 8995909N
		Pueblo Nuevo	5	1.47	383426E- 8994796N
		San José Pucate	5	1.63	383134E- 9015205N
		San Miguel La Cocha	5	1.03	373995E- 8989834N
		Castillo Grande	2	0.61	385389E- 8995243N
		Castillo Grande	2	0.18	380328E- 8976876N
		Castillo Grande	2	0.52	388995E- 8975440N
		J. Crespo y Castillo	2	0.43	389173E- 8998273N
		La Morada	5	0.95	375599E- 9020745N
		Palo de Acero-Huamalíes	5	1.24	358621E- 8978570N
		Merced de Locro	5	1.23	380266E- 8985796N
		Dámaso Beraun	2	1.52	383365E- 8959585N
	Ucayali	Callería	5	0.61	395359E- 9071823N
		Padre Abad	5	0.66	546513E- 9001025N
		Padre Abad	1	0.31	444682E- 9015463N
		Irazola	2	0.53	445791E- 9023025N
		Bajo Shiringal	5	0.64	471720E- 9022636N
		Nuevo Tahuantinsuyo	5	1.47	470935E- 9013764N
		Balle Sagrado	5	0.84	459718E- 9026534N
		Huacamayo	5	0.92	455921E- 9005300N
South	Cusco	La Convención	4	0.20	447599E- 8576210N
Average				0.91±0.48	

Source: Prepared by the author using data from 1 Florida *et al.* (2019); 2 Florida *et al.* (2018); 3 Llatance *et al.* (2018); 4 Arévalo-Hernández *et al.* (2017); 5 COPAIN (2014).

Figures 3 and 4 shows the geospatial analysis using data interpolation with the IDW extension of ArcGIS, version ArcMap10.5, software created in the USA.

The high bioacummulation capacity of cadmium in the cacao beans (Figure 2A and 2B) is relevant and suggests that current standards should take into

account the natural level of cadmium in the soil as well as cacao genotipes, in order to establish average levels of cadmium, allowing the categorization of agricultural soil not contaminated with cadmium. In addition, it should not be confused with tolerable limits for derived or processed products in the commercialization of cacao beans (Pastor, 2017). Under current regulatory criteria, cadmium concentrations should be reformulated to classify soil, according to the cacao absorption capacity and the criteria proposed by Meter *et al*. (2019), who calculated a tolerable maximum limit for cadmium value in dry beans or raw cacao mass at 1.1 µg g^{-1}.

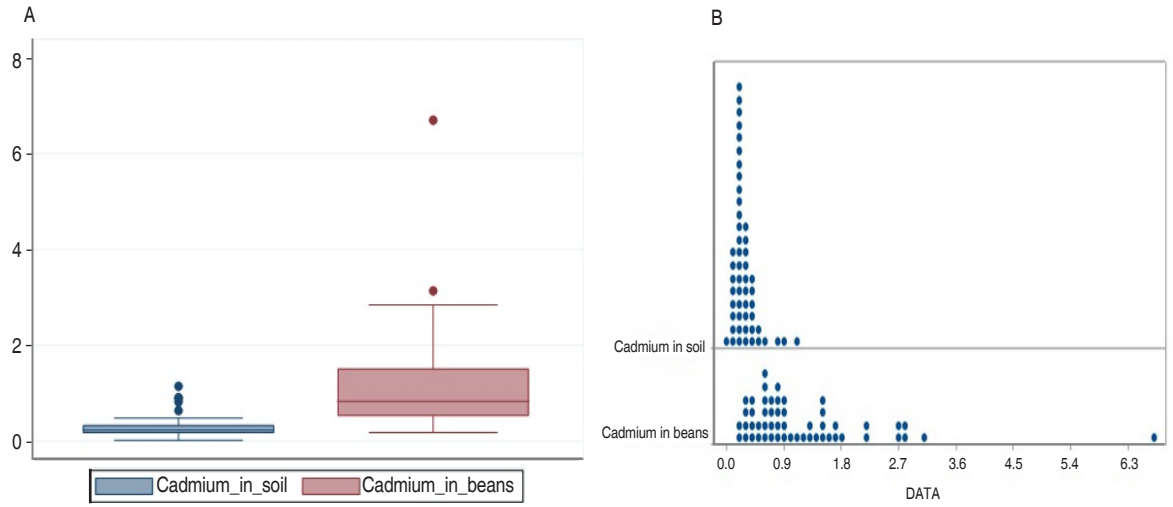

Figure 2. The dispersion of the available cadmium levels in the soil and the total cadmium in the cacao beans of the Peruvian territory. Diagram (A) and interquartile range (B).

Therefore, in the case of Peru, an uncontaminated soil should have a maximum of 0.36 µg g^{-1}; far below the limits set by the current USEPA standards (<0.43 µg g^{-1}), EU (<1 µg g^{-1}) and by the Peruvian Ministry of Environment (<1.4 µg g^{-1}) (Acevedo *et al*., 2005; Jiménez, 2015; Pastor, 2017). This would jeopardize the availability of areas for cacao production; however, it would prevent cacao production in heavily contaminated soils (Zug *et al*., 2019).

Data interpolation shows that the San Martin, Huánuco, Ucayali and Junín (central zone) and Tumbes (northern zone) regions have areas that exceed the tolerable limits as set by the EU; these areas represent 11.61% of the national territory (Figure 3). According to MINAGRI (2019) (acronym in Spanish) the Tumbes, Ancash and Pasco regions, together with other regions, contribute to only 3% of domestic production; therefore, they are not part of the calculations to measure the effects on future exports.

In addition, Figure 4 confirms the warnings of some researchers (Huamani *et al*., 2012; Arévalo-Hernández *et al*., 2017; Florida *et al*., 2018; Zug *et al*., 2019), since in central Peru, in 78.27% of San Martin, 87.74% of Huánuco, 6.49% of Ucayali, and 8.45% of the Junín region, there are areas with plantations that exceed the tolerable limits of cadmium, according to the EU, and these regions contribute to 79.2% of the domestic production (MINAAGRI, 2019); basic information for determining the effects on future exports.

Figure 4 shows geo-distribution by interpolating the total cadmium levels of beans from central Peru: in the San Martin region 78.27% of the total production areas have levels that exceed the limits that are tolerated by the EU, in Huánuco 87.74%, Junín 8.45% and Ucayali 6.49%.

Technologies for reducing cadmium levels in cacao beans

The geo-distribution (Figures 3 and 4) confirms that part of the Peruvian territory (central and northern zone) has beans with cadmium levels, which exceed the limits established by the EU, and similar to Peru, high levels

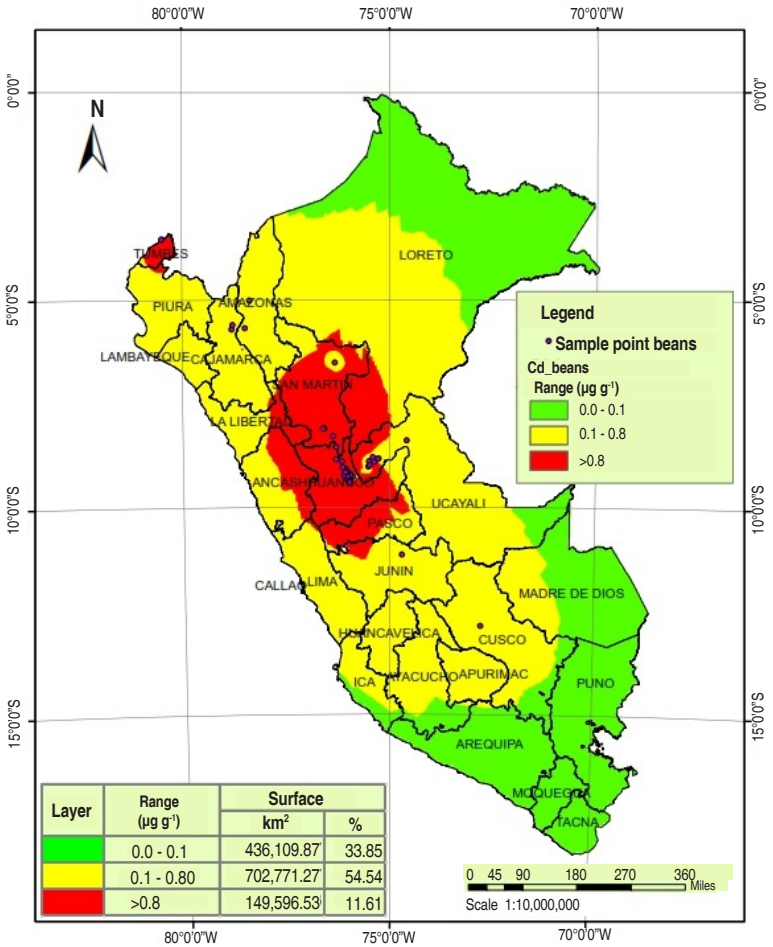

Figure 3. Geo-distribution of the cadmium in beans of the Peruvian territory.

have also been reported in parts of Ecuador (Mite *et al*., 2010; Chávez *et al*., 2015; Diaz *et al*., 2018; Argüello *et al*., 2019; Barraza *et al*., 2019), Venezuela (Sánchez *et al*., 2011; Lanza *et al*., 2016) , Colombia (Bravo *et al*., 2014; Jiménez, 2015; Rodríguez *et al*., 2019), Brazil (Oliveira *et al*., 2019) and Bolivia (Gramlich *et al*., 2017) . These countries contributed to world production with 12% in 2012 and 17% in 2019 (Morales *et al*., 2012; MINAGRI, 2016; Arvelo *et al*., 2017; Antolinez *et al*., 2020).

Cadmium in cacao is a challenge to overcome in the short term (Antolinez *et al*., 2020), therefore, appropriate technologies are needed to reduce cadmium levels in the beans and protect the product quality, as well as consumer health (Casteblanco, 2018; Engbersen *et al*., 2019; Maddela *et al*., 2020). In addition, the availability of the cadmium in the soil and its bioaccumulation in beans is influenced by natural concentration (Argüello *et al*., 2019), physical, and chemical factors of the soil, which can maintain control of metal mobilization and bioaccumulation (Prieto *et al*., 2009; Sanchez *et al*., 2011; Bravo *et al*., 2014; Arévalo-Gardini *et al*., 2017; Pereira *et al*., 2017; Florida *et al*., 2019) as well as the genotype of cacao grown (Arévalo-Hernández *et al*., 2017; Chupillon-Cubas *et al*., 2017; Barraza *et al*., 2019; Engbersen *et al*., 2019).

In this context, this research suggests that the most promising strategies to reduce cadmium in cacao beans its absorption by trees, adding soil amendments to alter soil characteristics, thus reducing the bioavailability of cadmium (Argüello *et al*., 2019; Meter *et al*., 2019). These agronomic techniques

offer advantages because of their low cost and minimal environmental impact as compared to other remediation procedures (Mohamed *et al*., 2017). Moreover, these techniques have shown favorable results along with the application of organic, inorganic, and combined fertilizers. In this regard, at the laboratory level was demonstrated, that the retention and mobility factor of cadmium depend on the quality of organic matter. In Colombia, Bravo *et al*. (2014) found significant effect in these processes, in addition, a better quality causes a lower cadmium mobility, avoiding contamination and toxicity through bioaccumulation. Furthermore, Ramtahal *et al*. (2019) applied biochar and lime *in vitro* and found that the two amendments were complementary in their action and they can be used to reduce cadmium bioaccumulation. There are already successful experiences, including Florida *et al*. (2019) in Padre Abad, Peru, who applied compost and NPK and found a significant reduction in cadmium levels of soil for cacao production, as well as the beans. Also, in Venezuela, Sánchez *et al*. (2011) applied phosphorus doses and the amount of available cadmium decreased because of the phosphorus effect.

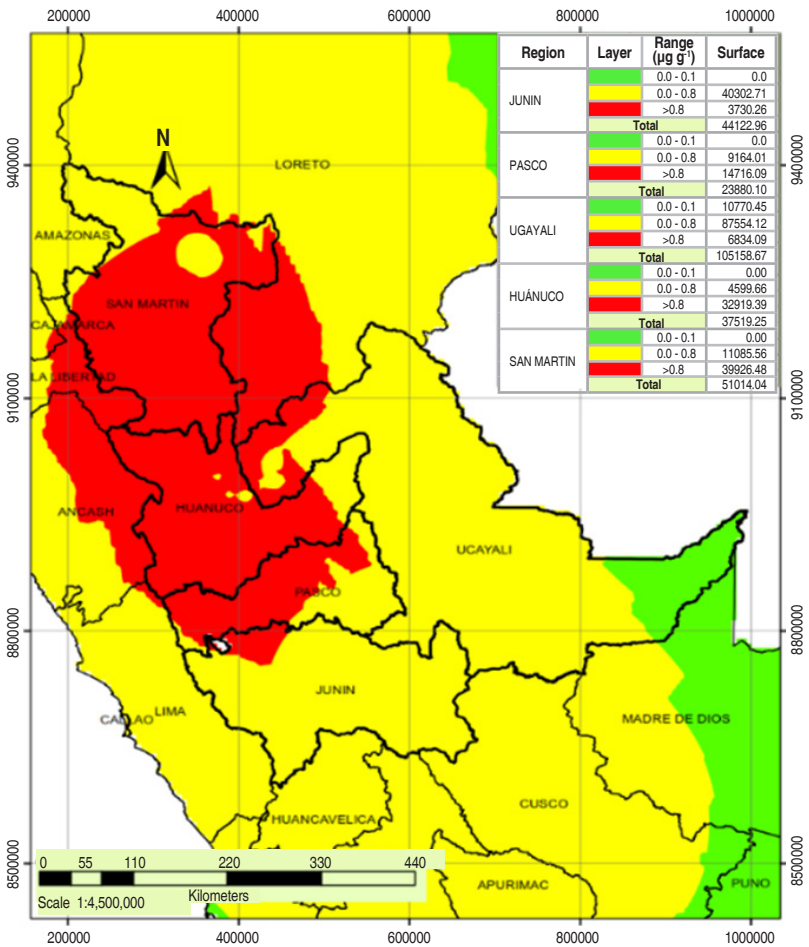

Figure 4. Regional area which exceeds the EU's tolerable limits.

The use of amendments from organic and mineral origin, together with efficient microorganisms, showed better results. In Colombia, Cáceres and Torres (2017) found changes in the diversity of the microbial community of the soil associated with cacao cultivation, as well as, potential cadmium tolerant strains that can be used in on-site bioremediation programs or microorganisms can be used in order to produce biotechnological products. Revoredo

and Hurtado (2017) evaluated the bioremedial activity of 3 strains of Streptomyces: *Streptomyces variabilis* (AB5 and X) and *Streptomyces* sp. (C2) in cacao plants using concentrations of 100 and 200 µg g^{-1} of Cd. The C2 strain showed bioremedial activity and reduced absorption by 39.67%. Also, Guerra-Sierra *et al*. (2014) found that the *Aspergillus* and *Trichoderma* genres have high percentages of cadmium biosorption in a liquid culture medium of 98.6% and 96%, respectively. These potential capabilities were studied by Mohamed *et al*. (2017) who used organic amendments, compost, and microorganisms (bacteria, fungi, mycorrhizae) and demonstrated that the integration of compost and microorganisms has a positive effect on reducing the bioavailability of cadmium in the soil.

Agroforestry systems have also demonstrated positive influences on the decrease of cadmium in cacao beans. In this context, Gramlich *et al*. (2017) found lower cadmium contents in agroforestry systems than in monocultures, further noting that specific absorption capacities of crops are related to the genotype and age of the plants. In Huánuco, Peru, Zug *et al*. (2019) assessed the influence of land management and the diversity of nearby vegetation; the increased biodiversity of plant species was positively correlated with the cadmium content in cacao, which may make them promising measures to counteract cadmium contamination in regions with high cadmium content in the soil.

The selection of cacao genotypes that have low bioaccumulation capacities for use in cadmium contaminated soils is another alternative (Engbersen *et al*., 2019). In Ecuador, Barraza *et al*. (2019) found differences in isotopes 114 and 110 of cadmium for three different cacao genotypes. In the same country, Barraza *et al*. (2017) found average values of 1.21±0.87 and 0.89±0.64 µg g^{-1} for the CCN51 genotype and other Ecuadorian national varieties. In the San Martin, Peru, region, Chupillon-Cubas *et al*. (2017) found that the absorption of cadmium for six cacao genotypes could be used as a pattern, and thus, found differences in absorption levels for both the aerial and root parts of the plants, with mean values for the EET-400 genotype being 13.18 and 6.55, common cacao (12.98 and 2.81), CCN-51 (11.89 and 3.31), POUND-12 (10.30 and 3.38), SCA-6 (9.57 and 5.92), and IMC-67 (6.78 and 2.12 µg g^{-1}). Also, Lanza *et al*. (2016) found different mean values for the genotypes: HNF (2.09), PNF (1.9), PF (1.82), PFC (1.76), HF (1.74), PFM (1.57), HFC (1.1), and HFM with 0.95 µg g^{-1}. The use of genotypes with low heavy metal accumulation capacities could be an alternative in order to avoid bioaccumulation problems. Therefore, it is necessary to study the genotype indicative of each cacao producing region.

Phytoremediation with plant species that have the ability to accumulate and tolerate high concentrations of cadmium in harvestable tissue (Tariq and Ashraf, 2016) is another alternative to remove metal contaminants such as cadmium; through phytofiltration, phytostabilization, phytoextraction, phytolatization, and phytotransformation (Casteblanco, 2018). In this regard, some plant species with hyperaccumulative capacities have been identified, including sunflowers (*Helianthus annuus*), which are a hyperaccumulator for cadmium (Tariq and Ashraf, 2016); soybeans when up to 300 mg kg^{-1} of TiO_2 nanoparticles are added to the soil, obtained up to 400% µg of cadmium per plant (Singh and Byeong, 2016). However, the process can be optimized if plant species are combined with microorganisms. According to Ahemad (2015), endophytic bacteria associated with hyperaccumulating plant species promote the efficiency of the process through three mechanisms: increasing the root surface area and root hair production; increasing the availability of metals; and increasing the transfer of soluble metals from the rhizosphere to the plant. Guarino and Sciarrillo (2017) used *Acacia saligna*, *E. camaldulensis*, rhizobacteria and mycorrhizals, and they found that phytostabilization can occur in the soil with lead, cadmium and zinc. Also, Hashem *et al*. (2016) found that the arbuscular mycorrhizals *Glomus mosseae*, *Glomus intraradices*, *Glomus etunicatum* and *Bassia indica* can be used to decrease the dispersion of cadmium in the soil.

Effect on exports

Global cacao production increased from 2005-2018 by approximately 800 thousand t; it reached almost 4.6 million (Cunha, 2018). Meanwhile, Ecuador produced, in the same period, 118 to 260 thousand t and went from making up 3% to 6% of the world´s production, becoming fourth among cacao-producing countries, showing an annual growth of 9.8% (Cunha, 2018). Peru also showed sustained growth in cacao, with it being the sixth largest crop, in terms of area, that is harvested in 16 regions, 57 provinces and 259 districts (INEI, 2017).

According to Table 5, in the last 10 years the cultivated area has had an annual growth of 10.45%, so that by 2020 it is forecasted to reach an area of 219.8 thousand ha. Also, domestic production has had an annual growth rate of 14% and by 2020 it is forecasted to reach 169.86 thousand t, with a projected yield of 840 kg ha^{-1}; the latter is due to the sustained increase (5%) of the performance, which increased from 555 in 2010 to 820 kg ha^{-1} in 2019. This is a very positive aspect for Peruvian production, which contributes to approximately 2% of the world´s production and ranks nineth in the world (MINAGRI, 2019).

Table 5. Area and performance of Peru in cacao production.

Year	Cultivated area (thousand ha)	Growth of cultivated area (%) *	National production (thousand t)	National production growth(%) *	Performance (kg ha^{-1})
2009	66.3[b]		36.8[b]		
2010	77.2[b]	16.44	46.6[b]	26.63	555[b]
2011	84.2[b]	9.07	56.5[b]	21.24	604[b]
2012	91.5[b]	8.67	62.5[b]	10.62	671[b]
2013	97.6[b]	6.67	71.8[b]	14.88	683[b]
2014	106.6[b]	9.22	81.7[b]	13.79	736[b]
2015	121.3[b]	13.79	87.3[b]	6.85	766[b]
2016	125.58[b]	3.53	107.9[b]	23.60	720[b]
2017	143[a]	13.87	121.8[a]	12.88	759[a]
2018	160[a]	11.89	135.3[a]	11.08	800[a]
2019	199[a]	24.38	149[a]	10.13	820[a]
2020	219.8*		169.86*		840*
	Average	10.45*		14*	

* Calculated by the author. Source: [a] MINAGRI (2019); [b] MINAGRI (2016)

Table 6 shows that in the last 10 years the price has remained relatively steady (ICO, 2020) and Peruvian exports have grown on average by 11%, from 145.86 million in 2010 to 348.66 million in 2019 and a record 421 million can be reached by the end of 2020, as long as prices in the first months of 2020 remain stable (ICO, 2020), despite the validity of the pandemic.

Table 6. Behaviour of Peruvian cacao exports through 2020.

Year	National production (thousand t)	Average international price ($)	Exports (Millions $) *	Export growth (%) *
2010	46.6[c]	3.13	145.86	
2011	56.5[c]	2.98	168.37	15.43
2012	62.5[c]	2.39	149.38	-11.28
2013	71.8[c]	2.44	175.19	17.28
2014	81.7[c]	3.06	250	42.70
2015	87.3[c]	3.14	274.12	9.65
2016	107.9[c]	2.89	311.83	13.76
2017	121.8[b]	2.03	247.25	-20.71
2018	135.3[b]	2.30	311.19	25.86
2019	149[b]	2.34	348.66	12.04
2020	169.86*	2.48**	421.25*	20.82
Average Growth Rate				11%

* Calculated by the author. ** Average price January-April (ICO 2020). Source: [b] MINAGRI (2019); [c] MINAGRI (2016)

The high levels of cadmium identified by Peruvian research from areas were cacao has been produced for the past 10 to 15 years; plants of this age have higher cadmium concentration (Arévalo-Gardini *et al*., 2017; Florida *et al*., 2019; Zug *et al*., 2019). In addition, from 66.3 thousand ha in 2009 to 199 thousand ha in 2019, more than 66% of the nation's area, were young plantations with low cadmium levels, which are allowed by the EU. At the same time, the technique of mixing the product and the strict control of organic producers is practiced by those marketing the product; this, along with other aspects, reduces the levels of cadmium in the cacao beans. Therefore, the problem of high levels of cadmium in Peruvian cacao will begin to increase in the coming years, given that according to the INEI (2017), in the last decade, cacao was the second alternative crop to replace coca in Peru and it is reasonable that in the next decade, the growth rate of new areas will decrease and there will be an increase in plantations that exceed 10 years of age, and thus have high levels of cadmium.

Table 7 shows that these regions contributed to 79.2% of the domestic production. Therefore, the areas contaminated with cadmium in these regions represent 41% of the national production and affect 31.25% of the total volume of Peruvian exports to the EU; this represents 53.07 thousand t of cacao beans and 131,624 million dollars, which will have an affect on the following years if appropriate actions are not taken by the indicated sector.

Table 7. Effects on Peruvian cacao exports due to high cadmium levels.

Region	2020 National production (thousand t)	Regional production (%)	Regional production (thousand t)	Regional area where EU limits are exceeded (%) *	Regional volume affected (thousand t) *	2020 Total export earnings (Million $)
San Martin	169.86	40.90	69.47	78.27	54.38	172 292.4
Junín		18.30	31.08	8.45	2.63	77 089.26
Ucayali		12.30	20.89	6.49	1.36	51 814.09
Huánuco		7.70	13.08	87.74	11.48	32 436.47
Cuzco		6.10	10.36	0.00	0.00	25 696.42
Ayacucho		5.00	8.49	0.00	0.00	21 062.64
Amazonas		4.70	7.98	0.00	0.00	19 798.88
Cajamarca		1.00	1.70	0.00	0.00	4 212.53
Pasco		1.00	1.70	0.00	0.00	4 212.53
Otros		3.00	5.10	0.00	0.00	12 637.58
Total		100	169.86	41		421 252.8
Average National Production Affected (thousand t)					69.83*	173 189.79*
Impact on Exports (76% destined for the EU)					53.07*	131 624.24*
Impact on Exports (%)					31.25*	

* Calculated by the author. Source: MINAGRI (2019)

FINAL CONSIDERATIONS

This research presented an analysis of research conducted in the major cacao-producing countries of South American. The available scientific data allowed determining that an average of 0.40 µg g^{-1} of available cadmium is found in the soil, classified as free of contamination by cadmium, according to the United States Environmental Protection Agency, which established a critical level at 0.43 µg g^{-1} of total cadmium in agricultural soil, and the EU, through the Kelley Directive, established a range of 0 to 1 µg g^{-1}. However, an average of 0.84 µg g^{-1} of total cadmium was found in the beans, which exceeds the EU standards and exceeds the levels found in the soil by 2.1 times; revealing a high bioaccumulation of metal in cacao beans from this region, thus, jeopardizing its quality and marketability to South America's the main export destination, the European Union.

In the case of Peru, 100% of the territory has soil that is not contaminated by cadmium (0.29 µg g^{-1}); however, it has high levels of cadmium in the beans (0.91 µg g^{-1}), concentrated in the central region, which affects approximately 31.25% of exports, representing a decrease of 131,624 million dollar per year. In Latin America, Ecuador, has the soil with the highest cadmium levels, and Ecuador and Venezuela the highest averages of cadmium in the cacao beans.

Significant advances are being made in technology in order to reduce bioavailability and bioaccumulation in the beans through the application of amendments (organic, inorganic and combined), agroforestry systems, bioremediation, and genotype selection. In addition, proposals have been made to reformulate the criteria to establish the maximum cadmium levels in raw beans at 1.1 µg g^{-1}, and a final proposal by the researchers is to redirect exports to markets that do not have limits for this metal, as a short term alternative.

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Short article

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Coverage effect on hardening of arrow cane (*Gynerium sagitatum* Aubl.) micropropagated plants

Efecto de cobertura en el endurecimiento de plantas micropropagadas de caña flecha (*Gynerium sagitatum* Aubl.)

https://doi.org/10.15446/rfnam.v74n2.87294

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ABSTRACT

To evaluate the effect of plastic film coverage on *ex vitro* acclimatization of arrow cane (*Gynerium sagitatum* Aubl.), shoots of "Criolla", "Martinera" and "Costera" cultivars were *in vitro* micropropagated in a medium supplied with 6-Benzylaminopurine and half of them were rooted in a medium with 1-Naphtalene acetic acid. Rooted and unrooted shoots were transplanted in a shade house with fog irrigation, into plastic trays (72 clusters per tray), using peat as substrate and half of them was covered with translucent plastic film during 5 days after transplant while the other half was maintained uncovered. The experiment consisted of a three-way factorial arrangement with 12 treatments distributed with a split-plot design where tray coverage was the main plot, cultivars were the split, and rooting condition was the split-plot. Each treatment (36 clusters) was repeated three times for a total of 1296 experimental units. After 40 days in the shade house, the survival rate was calculated, and plant heigth, number of shoots and number of roots data were analyzed by ANOVA (P<0.05) and means were separated by Tukey test (P<0.05). Plant survival was complete (100%) regardless of genotype, rooting, or coverage condition. Transferring plant into uncovered trays statistically resulted in higher levels for plant height, number of shoots, and number of roots. *Ex vitro* adaptation of micropropagated arrow cane plants without plastic film covers increased plant growth and reduced labor.

Keywords:
Ex vitro
Micropropagation
Plastic film cover
Poaceae
Transplanting

RESUMEN

Para evaluar el efecto de cubiertas de plástico en la aclimatación *ex vitro* de plantas micropropagadas de caña flecha (*Gynerium sagitatum* Aubl.), brotes de los cultivares "Criolla", "Martinera" y "Costera" fueron multiplicados en un medio con 6-Bencilaminopurina y la mitad de ellos posteriormente enraizados en un medio con 1-Acido Naftaleno acético. Ambos grupos de plantas fueron trasplantados bajos una polisombra, en bandejas plásticas (72 clústeres por bandeja) conteniendo turba como sustrato, la mitad con cubierta plástica transparente durante 5 días y la otra mitad sin cubierta. El experimento consistió en un arreglo de tres factores con 12 tratamientos distribuidos con un diseño de parcelas subdivididas, donde la cubierta fue la parcela principal, el cultivar la subparcela y la condición de enraizamiento la sub-sub parcela. Después de 40 días del trasplante, se calculó la tasa de supervivencia. Los datos de altura de planta, número de brotes y número de raíces fueron analizados por medio de ANOVA (P<0,05) y los promedios separados con la prueba de Tukey (P<0,05). La supervivencia de las plantas fue total (100%) independientemente de la cubierta, el genotipo y el enraizamiento de los brotes. La transferencia en bandejas sin cubiertas resultó en alturas de planta, y número de tallos y raíces estadísticamente mayores. La adaptación *ex vitro* de plantas micropropagadas de caña flecha en bandejas sin cubierta de plástico incrementó el crecimiento de las plantas y redujo la mano de obra.

Palabras clave:
Ex vitro
Micropropagación
Cubiertas de plástico
Poaceae
Trasplante

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Received: July 10, 2020; Accepted: December 17, 2020
Rev. Fac. Nac. Agron. Medellín 74(2): 9517-9522. 2021

ISSN 0304-2847 / e-ISSN 2248-7026

Arrow cane (*Gynerium sagitatum* Aubl.) is a Poaceae Indian native plant species that grows in the Americas from México to Paraguay associated to river shores, floodplains, and swamps where it behaves as colonizer for forest succession. The plant is a giant grass with erect stems that grows up to 8 m height. The upper part of the culms is formed by unfold leaf blades with an open fan-shaped form. Leaves are 160 to 230 cm long and 8 to 14 cm wide; a single culm can have up to 200 leaves during lifetime, keeping 19 to 28 fresh blades at any time. Underground, the plant forms leafy rhizomes that originate from old culms, becoming a profuse interconnected net that works, preventing soil erosion (Suárez, 2020). In Colombia, arrow cane is found in the shores of Cauca and Magdalena Valley rivers, Cauca, Valle del Cauca, and Antioquia departments. The plant is well adapted to the Colombian Atlantic Coast environmental conditions where it grows in the plains of Córdoba and Sucre departments where it is cultivated by aboriginal communities (Suárez, 2020). The central leaf nerve is the fiber source for local indigenous artisans to make crafts such as rings, shoes, necklaces, wallets, and the most famous, "*Sombrero Vueltiao*", which is a Colombia´s Cultural Symbol (GRIN, 2018; López and Suárez, 2018).

Micropropagation is a clonal technique used to propagate plants in closed containers, provided with artificial media, under controlled environmental conditions, and in abscense of microbes (Suárez, 2020). As part of a biotechnology program on arrow cane developed at the Institute for Applied Biotecnology in the Caribbean (IBAC), micropropagation has been used to massively propagate arrow cane plants using *in vitro* culture of explants with pre-existing meristems, a five-stages protocol that comprises mother plants preparation, *in vitro* establishment of explants, propagule multiplication, *in vitro* rooting of multiplied shoots and transfer to *ex vitro* conditions (Suárez *et al*., 2013). Improvements of the arrow cane micropropagation protocols include protocols for different cultivar in conventional semisolid media (Suárez *et al*., 2009; Pastrana and Suárez, 2009; Suárez *et al*., 2020a) or alternate double-phase system (Semisolid –liquid) to increase cost and time efficiency (López and Suárez, 2018). In both cases, micropropagated plants, *in vitro* rooted or not, have been successfully acclimatized to *ex vitro* conditions using different substrates (Suárez *et al*., 2020b).

Ex vitro adaptation is intended to secure survival for micropropagated plants, since *in vitro* culture conditions in closed recipients and high water saturation hinder the development of mechanisms to control water loss, such as cuticle accumulation and stomatal closure in leaves, a response associated with abscisic acid (ABA) levels in plant tissues (Suzuki *et al*., 2016). Micropropagated plants should be initially maintained under highly humid environments to avoid rapid dehydration when transferred to *ex vitro* conditions. Usually, micropropagated plants are transferred into plastic trays using a high retention water substrate (peat) and covered with transparent plastic films that allow light incidence while retain humidity for several days, respectively. Several plant species have demonstrated to resist water loss without plant coverage when transferred to *ex vitro* conditions (Kodym and Leeb, 2019). For arrow cane micropropagation, conventional *ex vitro* adaptation procedures comprises coverage during five days of recently transferred plants with transient removal of the covers every 2 h during daytime to avoid heating the plants, followed by spraying with distilled water; which increases the operational labor costs. In the present research, the survival and growth performance during *ex vitro* adaptation of arrow cane micropropagated plants with no coverage were compared to the conventional *ex vitro* adaptation protocol to evaluate the possibilities of avoiding plastic coverage and increasing cost efficiency (Bhojwani and Dantu, 2013; Gil *et al*., 2017; Makowczyńska *et al*., 2016).

MATERIALS AND METHODS

The plant material consisted of micropropagated plants of arrow cane "Criolla", "Martinera" and "Costera" cvs. Plants were grown *in vitro* from 2-3 cm stem segments with a single axillary meristem. Explants were disinfected for 15 min in a 1.25% NaClO (sodium hypochlorite) solution and rinsed three times with sterile-distilled water inside a laminar flow hood. Surface-sterilized explants, a group of three shoots each, were established in 250 mL polycarbonate flasks dispensed with 30 mL of the establishment medium consisting of semisolid MS salts (Murashige and Skoog, 1962) with (in mg L^{-1}) myo-

inositol (100), thiamine HCl (0.4), sucrose (30,000) and Agar (8,000) (Sigma Co®). Flaks were established with a single explant, covered with two layers of heavy duty aluminum foil, sealed with Parafilm®, and stored at 20 °C with 12 h of photoperiod (40 µmol m^{-2} seg^{-1}) with subcultures every six weeks to fresh medium of the same formulation. After four subcultures, established shoots were used for the next stage.

Shoot proliferation stage was performed under the same conditions indicated for the establisment stage but the medium was supplied with 0.5 mg L^{-1} BAP (6-Benzylaminopurine). After three subcultures, aproximately 250 cultures were transferred to a fresh medium supplied with BAP and 250 were transferred to a medium deprived of BAP but supplied with 0.5 mg L^{-1} NAA (1-Naphtalene acetic acid) for the *in vitro* rooting. Explants in multiplication and rooting media were maintained during six weeks before *ex vitro* transfer.

Transfer to *ex vitro* conditions

In vitro multiplied shoots (200) and *in vitro* rooted shoots (200) were maintained in culture for six weeks and then, they were removed from the recipient, washed with sterile water to eliminate medium residues, and transferred into 72-plug containers filled with peat as substrate. Trays were placed in a shade house with a 50% light under two different *ex vitro* conditions at the Plant Biotechnology Lab of the Universidad de Córdoba (Montería – Colombia 8°75'N and 75°52'W, 14 masl, 28 °C and 82% R.H.). Trays with half number of shoots (rooted and rootless) were covered with plastic transparent film during 5 days with periodical removal of covers every 2 h, and spraying with sterile water during every removal; after 5 days, covers were removed, and plants were fog-irrigated three times per day during 2 min each. Simultaneously, trays with the 50% remaining plants (rooted and rootless) were maintained uncovered but fog-irrigated three times per day for 2 min each. Trays with transferred plants were maintained under shade house conditions for 40 days. Temperature in the shade house was 28 °C average. The experiment was a factorial with three factors [coverage (2), cultivars (3) and rooting condition (2)], and 12 treatments distributed with a split-plot design; where coverage was the main plot, genotype was the split and rooting condition the split-plot. Each treatment (half tray = 36 clusters) was repeated three times for a total of 1296 experimental units. After 40 days, the survival rate was calculated, the number of shoots per plant, shoot height and number of roots per plant were analyzed using an ANOVA (α= 0.05) based on the model $Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + C_k + (AC)_{ik} + (BC)_{jk} + (ABC)_{ijk} + E_{ijk}$; where A_i was the effect of the coverage, B_j was the effect of the genotype, C_k was the effect of the rooting condition, AB the effect of the whole plot error and *E* the split plot error. Means were separated using a Tukey test (α=0.05).

RESULTS AND DISCUSSION

The arrow cane explants from "Criolla", "Martinera" and "Costera" cvs were *in vitro* established, shoots proliferated, half of them rooted, and both rooted and unrooted, were transplanted to *ex vitro* conditions (Figure 1-A, B, C).

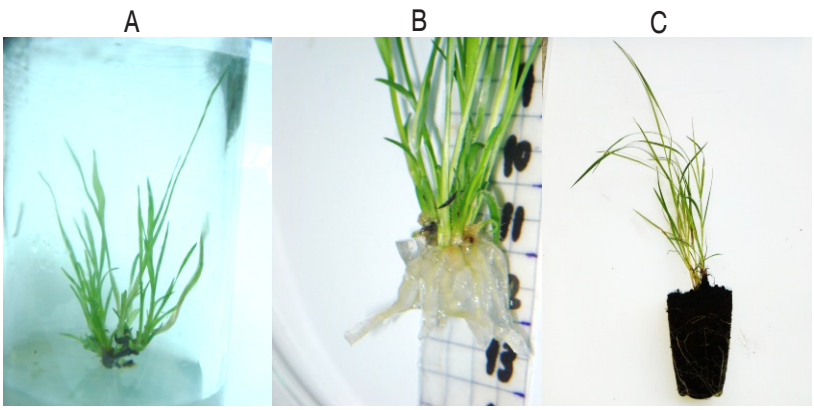

Figure 1. Cultures of arrow cane "Criolla" cv. in stages of propagule multiplication (A), *in vitro* rooting (B) and *ex vitro* acclimatization (C).

The transfer to *ex vitro* conditions occurred either under plastic coverage during 5 days period after transplanting or without cover (Figure 2A-B); however for both cases, the plants showed a complete survival rate regardless of the rooting condition (rooted or rootless) of transferred material. The *ex vitro* adapted plants showed normal growth and complete adaptation to open conditions (Figure 2C).

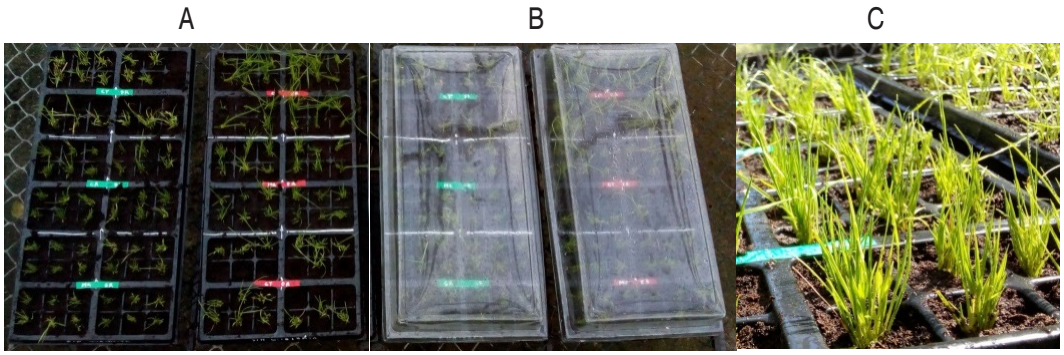

Figure 2. *Ex vitro* adaptation of arrow cane (*Gynerium sagitatum* Aubl.) micropropagate plants. A: uncovered transplanted plants, B: plastic-covered transplanted plants, C: acclimatized plants.

The ANOVA allowed to evidence that coverage statistically affected (P<0.05) plant height, number of shoots, and number of roots variables. The Tukey mean separation test showed that plants in trays with no plastic coverage significantly increased height, number of shoots, and number of roots compared to plants covered during 5 days period after transplanting (Figure 3).

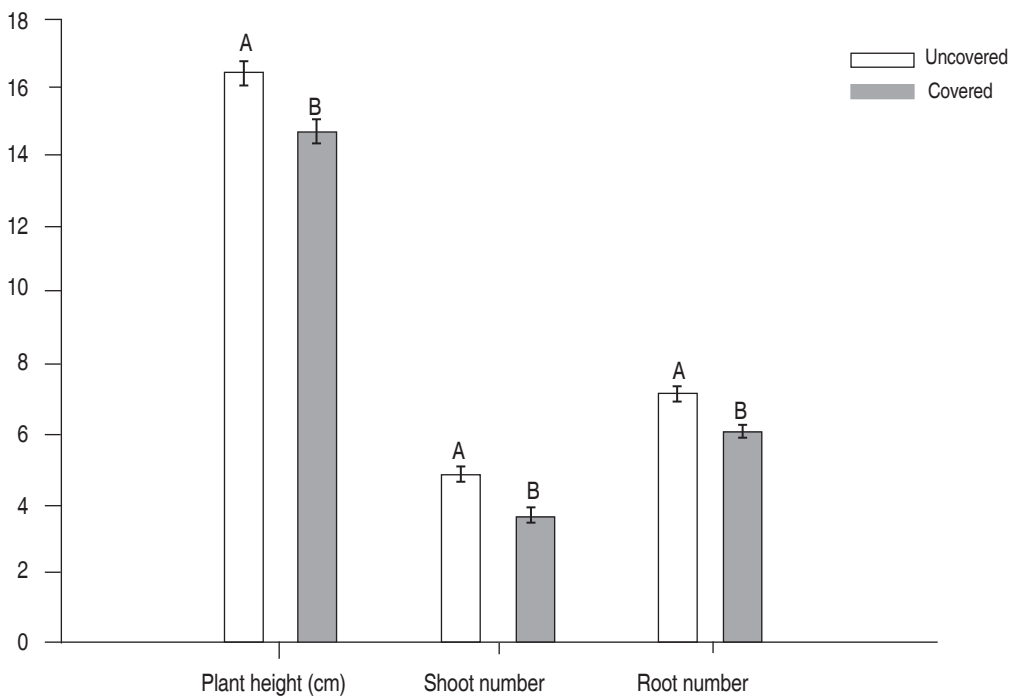

Figure 3. Effect of plastic coverage on plant growth factors during *ex vitro* acclimatization of arrow cane (*Gynerium sagitatum* Aubl.) micropropagated plants.

When uncovered, the *in vitro*-rooted "Costera" and "Martinera" plants showed a significant increase ($P<0.05$) in plant height compared to adapted plants from transferred rootless shoots; however, "Criolla" plants rooted or rootless at the transplant stage were not different concerning plant height. For all three cultivars, plants were not different in number of shoots despite rooted or rootless condition; and for number of roots, only "Criolla" plants showed a significantly higher number of adventitious roots when plants were transferred from the *in vitro* rooting stage compared to those transferred from the rootless multiplication stage (Table 1). Among all cultivars maintained without plastic covers during the initial 5 days acclimatization phase, the *in vitro*-rooted "Criolla" plants showed the highest value in plant height, number of shoots and number of roots during the *ex vitro* adaptation stage.

When covered, adapted plants from rootless "Criolla", "Costera" and "Martinera" cvs were not different ($P>0.05$) with respect to plant height; however, based on the Tukey test rooted "Costera" plants had larger shoots than "Martinera" rooted plants. Rooting condition of the transferred propagule and genotype had no effect on number of shoots. Only "Martinera" plants transferred from rootless cultures showed a significant reduction of the number of adventitious roots when compared to rooted shoots of all three cultivars. Of all the covered plants, "Costera" transferred from *in vitro* rooting stage showed a higher plant height and more adventitious roots while "Martinera" and "Criolla" produced more shoots (Table 1).

Micropropagation is performed under controlled environmental conditions such as light, moisture and temperature that drastically change when plants are transferred to *ex vitro* (Yescas *et al*., 2016). The water-saturated atmosphere when plants are inside the culture recipient is significantly reduced when they are transferred to *ex vitro* in the acclimatization stage; therefore, mechanisms such as plant coverage with plastic film covers to reduce plant dehydration are usually implemented. Translucent plastic film is the most used cover material because of the cost, handling and recyclable use; however, heating inside the tray is a limiting factor that can result in heat stress for covered plants (Ilczuk and Jacygrad, 2016; Makowczyńska *et al*., 2016; Gil *et al*., 2017; Dreger *et al*., 2019). Plastic covers must be gradually moved to increase dehydration tolerance and to reduce temperature, a stage that can take long time for some species, which increases labor and costs (Indacochea-Ganchozo *et al.,* 2017; Suárez, 2020).

Table 1. Growth of arrow cane (*Gynerium sagitatum* Aubl.) micropropagated plants "Costera", "Criolla" and "Martinera" cvs transferred to *ex vitro* conditions with or without covers.

Variable	Uncovered					
	Rootless			Rooted		
	Costera	Criolla	Martinera	Costera	Criolla	Martinera
Plant hight (cm)	11.22 e	17.86 abcd	10.86 e	17.46 abdc	21.72 a	19.80 ab
Number of shoots	5.40 ab	4.40 ab	4.20 ab	4.60 ab	6.40 a	4.20 ab
Number of roots	2.80 cd	6.60 bc	5.00 bcd	7.00 bc	12.60 a	8.80 ab
	Covered					
Plant hight (cm)	13.20 cde	16.92 abcde	12.06 cde	18.10 abc	15.40 bcde	11.92 de
Number of shoots	2.60 b	4.40 ab	2.60 b	2.80 b	4.60 ab	4.60 ab
Number of roots	6.00 bcd	5.20 bcd	1.80 d	8.40 ab	7.40 b	7.40 b

Different letters between columns mean statistically significant differences.

In the present research, plants transferred to *ex vitro* conditions under plastic coverage showed lower plant height, less number of shoots and roots compared to those transferred and acclimatized without coverage. For the conditions of the present research, *in vitro* propagation was performed at 20 °C and in the shade house, *ex vitro* temperatures were at least 6-8 °C higher; however, for plants that were acclimatized under plastic covers during a 5 days period after transplanting, temperatures may were even higher. Under those conditions, the covered plants probably increased respiration rates, resulting in a higher consumption of energy reserves than the

uncovered plants for the same period of time (Fahad *et al*., 2017). Due to micropropagated plants are unable to readily photosynthesize after transfer to *ex vitro* conditions and are completely dependent on accumulated organ reserves, less heat-stressed uncovered plant may have more energy reserves available for growth than covered ones, and can develop a higher number of shoots, roots and larger plants (Kodym and Leeb, 2019). Although the effect of respiration rates and temperatures inside the covered trays were not evaluated in this research, such evaluations are strongly suggested for further studies.

CONCLUSION

The transferring of arrow cane "Criolla", "Martinera" and "Costera" cvs micropropagated plants with no plastic coverage allowed a complete (100%) adaptation to *ex vitro* conditions, increased the plant height, the number of shoot, the number of roots and reduced the labor and plant handling during stage IV of micropropagation.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the Instituto de Biotecnología Aplicada del Caribe (IBAC) and the Universidad de Córdoba for their support.

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Revista Facultad Nacional de Agronomía

Control and translocation of saflufenacil in fleabane (*Conyza* spp.) according to plant integrity

UNIVERSIDAD NACIONAL DE COLOMBIA

Control y translocación de saflufenacil en yerba canicera (*Conyza* spp.) en función de la integridad de la planta

https://doi.org/10.15446/rfnam.v74n2.90951

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Keywords:
Conyza spp
Herbicide mobility
Regrowth

ABSTRACT

Efficient herbicide absorption and translocation, and satisfactory weed control can be affected by the site of herbicide application. However, during harvesting of crops of previous soybean sowing, the cutting process made by harvesters on the fleabane may generate a difficult management in pre-sowing of the crop by limiting the leaf area of the absorption of the herbicide. Experiments were conducted to evaluate the control efficiency and translocation potential of saflufenacil in fleabane plants with different leaf and stem conditions. Experiment I was arranged in a 2x10 factorial scheme, with factor A corresponding to leaf integrity, and factor B corresponding to different levels of injury and saflufenacil application. Weed control was evaluated at 7, 14, and 21 days after herbicide treatment (DAT), and dry matter was evaluated at 21 DAT. Experiment II consisted of applying saflufenacil to different fleabane structures, where the percentage of necrotic area was evaluated at 1, 3, 5, and 7 DAT. Fleabane control was higher than 75% in all treatments with saflufenacil application, with greater control in plants previously defoliated. Saflufenacil application on 10 and 20 cm hairy fleabane plants was also efficient in all treatments. Saflufenacil application in old stem showed a larger necrotic area, while application in the site of the cutting resulted in a lower necrotic area. The main pathway for translocation of saflufenacil is via xylem and the stem proved to be the absorption element of the herbicide when leaf area is limited.

Palabras clave:
Conyza spp
Movilidad herbicida
Rebrote

RESUMEN

La eficiencia de absorción, translocación y control satisfactorio de malezas pueden ser afectados por el lugar de aplicación del herbicida. Sin embargo, durante la recolección de cultivos previos a la soya, el corte realizado por la cosechadora en la yerba carnicera dificulta el manejo de cultivos en pre-siembra al limitar el área foliar de absorción. Se realizaron experimentos para evaluar la eficiencia del control y el potencial de translocación de saflufenacil en plantas de yerba carnicera en diferentes condiciones. El primer experimento se organizó en esquema factorial 2x10. El factor A corresponde a la integridad de la hoja y el factor B corresponde a los diferentes niveles de daño y aplicación de saflufenacil. El control de *Conyz*a se evaluó a los 7, 14 y 21 días después del tratamiento herbicida (DAT), y el peso seco a los 21 DAT. El experimento II consistió en aplicar saflufenacil a diferentes estructuras de *Conyz*a, donde se evaluó el porcentaje de área necrótica a 1, 3, 5 y 7 DAT. El control de *Conyz*a obtenido fue superior al 75% en todos tratamientos usando saflufenacil, con mayor control en plantas previamente defoliadas. La aplicación de saflufenacil en plantas de 10 y 20 cm fue eficaz en todos los tratamientos. Además, saflufenacil aplicado en el tallo viejo mostró un área necrótica mayor, mientras la aplicación en el sitio del corte generó como resultado un área menos necrótica. La translocación de saflufenacil se da através del xilema y el tallo demostrando ser el elemento a través del cual se da la absorción del herbicida.

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Received: January 27, 2021; Accepted: March 09, 2021
Rev. Fac. Nac. Agron. Medellín 74(2): 9523-9530. 2021

ISSN 0304-2847 / e-ISSN 2248-7026

Species of *Conyza* spp. (hairy fleabane) are one of the main weeds found in soybean (*Glycine max* (L.)) and corn (*Zea mays*) in Brazil (Kaspary *et al*., 2016).The hairy fleabane has high rusticity, abundance in the seeds production and high potential regrowth; besides, the existence of biotypes resistant to the most used herbicides in the broad-leaves management (Osipe *et al*., 2013). These features contribute to the increased occurrence of these weeds in agricultural areas, which hinder their control.

Methods for integrated management of resistant weeds must be continuously carried out in production areas. In this context, the winter management is highlighted, since allows the control of plants before they produce seeds and feed the soil seed bank (Constantin *et al*., 2013). Autumnal management is a promising alternative in the context of limited postemergence herbicide options for *Conyza* spp. control, allowing its management in early stages or during regrowth (Dan *et al*., 2013). However, during harvesting of certain crops, the cutting made by harvesters on the fleabane may generate a difficult management in pre-sowing of the crop by limiting the leaf area of the herbicide absorption.

The use of protoporphyrinogen oxidase-inhibiting herbicides (PROTOX) such as saflufenacil has stood out as an alternative for managing glyphosate-resistant fleabane biotypes (Mellendorf *et al*., 2013). Saflufenacil belongs to the chemical group pyrimidinedione, and is recommended in preharvest desiccation, presowing burndown, and pre-emergence of dicotyledonous weeds (Grossmann *et al*., 2011). Due to rapid action on plant tissues, these herbicides usually have low translocation potential, being predominantly translocated via xylem, except for saflufenacil, which presents some mobility via phloem (Ashigh and Hall, 2010; Oliveira Júnior, 2011).

Efficient herbicide absorption, translocation and satisfactory weed control can be affected by the site of product application in plant structures, requiring applications at the correct stages and good leaf coverage, mainly when referring to contact herbicides (Trezzi *et al*., 2009). PROTOX-inhibiting herbicides can be absorbed by different plant structures, including roots, stems, and leaves. The contact place where the herbicide is applied can interfere the translocation and efficiency of the herbicide. Moreover, plant age and turgor and leaf surface characteristics, can influence herbicide absorption and translocation (Trezzi *et al*., 2009; Grossmann *et al*., 2011).

Impaired height and leaf area in *Conyza* spp. plants that have been cut in the harvest of winter crops, can be a limiting factor for adequate coverage of saflufenacil spray, making the control a difficult target. Nevertheless, the ability of this herbicide to penetrate and damage plants when in contact with the remaining stems in the area has scarce studies. Thus, the present study aimed to evaluate the control efficiency and translocation potential of the herbicide saflufenacil when applied in fleabane plants with different leaf and stem conditions.

MATERIALS AND METHODS

The study was conducted from January to April 2018 in a greenhouse, which consisted of two experiments. Plants from an area with a record of glyphosate resistance in the city of Santa Maria, Rio Grande do Sul, Brazil, were used in both experiments. Experimental units consisted of 5 L plastic pots filled with Gray-Brown Argisol. Each experimental unit contained a single *Conyza* spp. plant collected in the field at a standard height of 20 cm and immediately was transplanted. Plants were irrigated daily until the average height of 25 cm, when treatments were established in the different experiments.

Experiment I aimed to evaluate the efficacy of the saflufenacil herbicide in *Conyza* spp. plants with different injuries, and a experimental design was carried out in completely randomized design, with four replicates. Treatments were arranged in a 2x10 factorial scheme, in which factor A represented leaf integrity and factor B consisted of different levels of injury and saflufenacil application, according to Table 1. For factor B, cutting process was made using a scissors, and the different periods were used to obtain plants without regrowth (cutting at 1 Day before application (DBA)) and with regrowth (cutting at 8 DBA), simulating situations of loss of *Conyza* spp. shoots during harvest.

The herbicide saflufenacil was applied at a rate of 35 g (active ingredient) a.i ha^{-1}, with addition of 0.5% mineral oil, at the time indicated in the Table 1. Treatments were applied with a CO_2 pressurized backpack sprayer

equipped with 110.015 nozzles and calibrated to provide an application volume of 150 L ha^{-1}. The variables analyzed were controlled at 7, 14, and 21 days after herbicide treatment (DAT), in a percentage scale where zero (0) represents the absence of injuries, and one hundred (100) represents complete plant death. At 21 DAT, shoots were collected and placed in a greenhouse with forced air circulation at 65 °C for three days to determine the dry matter (DM) of fleabane plants.

Table 1. Treatments related to Experiment I arranged in a factorial scheme.

Factor A **Leaf Integrity**	With leaves Without leaves
Factor B **With/Without cutting and** **Saflufenacil application**	Whole plant Cutting 10 cm 8 DBA[1] Cutting 20 cm 8 DBA Cutting 10 cm 1 DBA Cutting 20 cm 1 DBA Whole plant + SAFL[2] Cutting 10 cm 8 DBA + SAFL Cutting 20 cm 8 DBA + SAFL Cutting 10 cm 1 DBA + SAFL Cutting 20 cm 1 DBA + SAFL

[1] DBA=Days before application of saflufenacil. [2] SAFL=Saflufenacil.

Experiment II aimed to evaluate the translocation capacity of the herbicide saflufenacil when applied in different places in fleabane plants.It was conducted in a completely randomized design, with three replicates. Treatments consisted of the localized application of 35 g a.i. ha^{-1} of the herbicide saflufenacil, with an addition of 0.5% mineral oil, in the following plant structures: leaf located close to the apex (new leaf); leaf located close to the base (old leaf); stem located close to the apex (new stem); stem located close to the base (old stem); and the region of the cut in 10 cm plants. The herbicide was deposited on plant structures using a flexible brush in a region of approximately 1.5 cm at the end of the day (7:00 PM). The variable analyzed was the percentage of necrotic area in fleabane plants at 1, 3, 5 and 7 DAT.

To measure the percentages of necrotic area, fleabane plants were photographed in all experimental units at each evaluation period. The software ImageJ® was used for image processing, where green plant parts were selected. Subsequently, non green parts were suppressed from the image, which was considered to be necrotic areas due to the action of the herbicide (Figure 1). Afterward, the percentage of the necrotic area was calculated by the difference between the total plant area and the green area, that means, without necrosis symptoms from herbicide activity, according to Ramos *et al.* (2015).

The collected data were subjected to analysis of variance by the F test ($P<0.05$). When statistically significant, the means were compared by the Tukey test ($P<0.05$) using R software (R Core Team, 2020).

RESULTS AND DISCUSSION

Analysis of variance showed statistical significance for all variables analyzed in both experiments. In experiment I, fleabane control at 7 DAT was higher than 75% in all treatments with saflufenacil application, differing from the other treatments without applying of the herbicide (Table 2). Saflufenacil proved to be an efficient alternative in the control of glyphosate-resistant fleabane biotypes and acetolactate synthase (ALS)-inhibiting herbicides, and can be mixed with herbicides with other mechanisms of action (Dalazen *et al.*, 2015; Davis *et al.*, 2010).

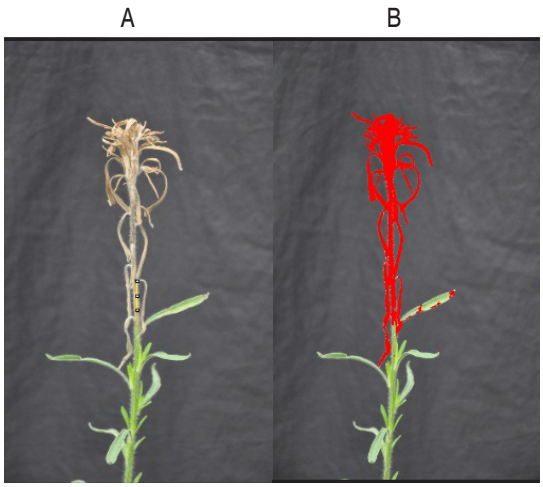

Figure 1. Identification and quantification of the percentage of the necrotic area of fleabane plants (*Conyza* spp.) after localized application of saflufenacil, indicating the original image without manipulation (A) and the selection of damage areas underlined in red color (B).

Comparing the leaf integrity of plants, the treatments in which plants were previously defoliated showed a higher control percentage at 7 DAT, except for the treatment with cutting+herbicide of 10 cm plants at 1 DBA (Table 2). Treatments with and without leaves, the most significant weed controls (higher than 90%) were observed when saflufenacil was added to treatments on defoliated plants. Moreover, approximately 20% higher control was achieved by applying the herbicide to whole plants (uncut) without leaves compared to treatment with leafy plants. This result is related to the deposition of saflufenacil on the stem of fleabane plants due to the absence of leaves. Thus, once absorbed in the stem, saflufenacil is translocated to the other parts of the plant, acting on the chlorophyll synthesis pathway (Oliveira Júnior, 2011). The green stem of fleabane plants stands out regarding the presence of this photosynthetic pigment, which can favor the mode of action of the herbicide.

The results showed that saflufenacil application on cut-leaf plants, simulating the harvest of winter cereals, was higher to the treatment of uncut-leafy plants at 7 DBA (Table 2). However, defoliated cut plants showed lower controls when compared with treatments mixed with herbicides. In this sense, fleabane plants have a high regrowth capacity after management practices with potential for recovery (Oliveira Neto *et al*., 2013).This regrowth capacity highlights the need to adopt alternatives that contribute to chemical and mechanical control through other practices such as crop management (Oliveira Neto *et al*., 2010).

At 7 DAT, the treatment with the cutting of 10 cm plants at 1 DBA plus herbicide application did not differ between plants with and without leaves, showing satisfactory control (93%). This shows greater sensitivity of 10 cm fleabane plants for saflufenacil application (Table 2). Also, 20 cm plants that were cut and received saflufenacil did not differ from whole plants that received the herbicide, regardless of leaf integrity (Table 2). Growth and development characteristics must be observed for proper control of *Conyza* spp. through saflufenacil. Higher plants in an advanced growth stage have a high capacity for lateral shoot emission to recover from herbicide exposure (Moreira *et al*., 2010). However, this study showed reasonable control of *Conyza* spp. plants up to 25 cm height.

After 14 and 21 DAT, the application saflufenacil in *Conyza* spp. plants of 10 and 20 cm was efficient in all treatments, with control equal to or greater than 98% (Table 2). Treatments without saflufenacil application generally showed a decrease in fleabane mechanical injuries from 14 DAT, differing from herbicide treatments and indicating plant recovery (Table 2).

Table 2. *Conyza* spp. control (%) in different tissue conditions at 7, 14, and 21 days after application (DAT) of saflufenacil.

Treatment	7 DAT		14 DAT		21 DAT	
	With leaves	Without leaves	With leaves	Without leaves	With leaves	Without leaves
Whole plant	0 eB	53 bA	0 dB	48 bA	0 cB	33 cA
Cutting 10 cm 8 DBA[1]	24 dB	64 bA	16 cB	56 bA	10 cB	41 bcA
Cutting 20 cm 8 DBA	16 dB	61 bA	8 cdB	49 bA	8 cB	35 cA
Cutting 10 cm 1 DBA	55 cB	65 bA	46 bA	48 bA	38 bA	48 bA
Cutting 20 cm 1 DBA	14 deB	62 bA	12 cB	50 bA	9 cB	47 bA
Whole plant + SAFL[2]	76 bB	95 aA	95 aA	100 aA	98 aA	100 aA
Cutting 10 cm 8 DBA + SAFL	88 aB	97 aA	97 aA	100 aA	99 aA	100 aA
Cutting 20 cm 8 DBA + SAFL	83 abB	96 aA	93 aB	100 aA	98 aA	100 aA
Cutting 10 cm 1 DBA + SAFL	93 aA	93 aA	99 aA	100 aA	100 aA	100 aA
Cutting 20 cm 1 DBA + SAFL	85 abB	96 aA	99 aA	100 aA	100 aA	100 aA
C.V.(%)[3]	8.11		7.50		9.59	

Means followed by the same uppercase letter in the row, comparing leaf integrity for each evaluation period, and by the same lowercase letter in the column, comparing injury levels for each treatment, indicate no significant difference by the Tukey test (P<0.05). [1]DBA=Days before application of saflufenacil. [2]SAFL=Saflufenacil. [3]Coefficient of variation.

When associating saflufenacil and glyphosate, a similar study showed satisfactory control (97%) at 14 DAT of *Conyza bonariensis* plants with regrowth and glyphosate resistance in an apple orchard (Pereira *et al*., 2016). Furthermore, mechanical weed control of *Eryngium horridum* with 10 to 15 cm mowing in natural pasture removed the shoots of weeds; however, it did not reach lateral buds, allowing regrowth at 20 DAT (Pellegrini *et al*., 2007), a similar situation was observed in the present study.

Treatments with saflufenacil application decreased DM in comparison to those that have not applied herbicide in whole plants with or without leaves (Table 3). The DM of whole leafy plants decreased by 64% after saflufenacil application compared to an entire leafy plant without herbicide treatment. Cesco *et al*., (2019) evaluated *Conyza* spp. control at different stages of plant development, the saflufenacil rate of 60 g a.i. ha^{-1} decreased DM by approximately 77 and 54% in fleabane plants up to 5 and 20 cm, respectively, and led to a low regrowth percentage in plants up to 20 cm height.

In treatments with saflufenacil application, DM differed only between whole plants with and without leaves, with lower DM in defoliated plants (Table 3). Besides, defoliation of *Conyza* spp. plants decreased DM approximately by 50 to 70%, in the control treatment (whole leafy plant) and in treatments in which the plants had regrowth (cutting at 8 DBA, without herbicide application), showing statistical difference (Table 3). The results indicated the efficiency of saflufenacil in controlling *Conyza* spp. plants with injured leaves, for example, after harvesting damage. Moreover, the control and reduction of DM in defoliated *Conyza* spp. plants may be related to herbicide absorption in the plant stem.

Treatments with saflufenacil application on leaves (new and old ones) showed a necrotic area less than 20% in all evaluations (Table 4). Foliar applications of saflufenacil tend to concentrate in the meristematic regions of the plant and not translocate towards the roots (Budd *et al*., 2017). Furthermore, it is known that this herbicide has a weak acid character, with greater translocation via xylem and limited translocation via phloem (Grossmann *et al*., 2011), which may justify the low mobility from leaves. A

Table 3. Dry matter (DM) of *Conyza* spp. in different tissue conditions at 21 days after application (DAT) of saflufenacil.

Treatment	DM (g plant^{-1})	
	With leaves	Without leaves
Whole plant	7.72 aA	2.70 aB
Cutting 10 cm 8 DBA[1]	4.48 abA	1.70 abB
Cutting 20 cm 8 DBA	3.97 bcA	1.83 abB
Cutting 10 cm 1 DBA	1.28 cdA	0.75 bA
Cutting 20 cm 1 DBA	3.61 bcA	1.44 abA
Whole plant + SAFL[2]	2.77 bcA	0.55 bB
Cutting 10 cm 8 DBA + SAFL	1.06 cdA	0.30 bA
Cutting 20 cm 8 DBA + SAFL	1.51 cdA	0.45 bA
Cutting 10 cm 1 DBA + SAFL	0.40 dA	0.30 bA
Cutting 20 cm 1 DBA + SAFL	0.52 cdA	0.37 bA
C.V.(%)[3]	81.15	

Means followed by the same uppercase letter in the row, comparing leaf integrity for each evaluation period, and by the same lowercase letter in the column, comparing injury levels for each treatment, indicate no significant difference by the Tukey test (P<0.05). [1] DBA=Days before application of saflufenacil. [2] SAFL=Saflufenacil. [3] Coefficient of variation.

study on saflufenacil absorption and translocation in wheat (*Triticum aestivum* L.) found that the absorbed herbicide showed low translocation from treated leaves, corroborating the data observed in the present study (Frihauf *et al.*, 2010).

The lowest average necrotic areas were observed in the treatment where the herbicide was applied only in the region of the cutting, being lower to the other treatments at 3, 5, and 7 DAT, with a necrotic area less than 2% (Table 4).

Table 4. Necrotic area (%) of *Conyza* spp. evaluated at different times after localized application of saflufenacil.

Treatment	Necrotic area (%)			
	1 DAT	3 DAT	5 DAT	7 DAT
New leaf	0.42 b	11.44 c	15.15 b	18.45 b
Old leaf	5.13 ab	9.28 c	15.10 b	15.70 b
New stem	8.33 a	23.04 b	22.12 b	24.45 b
Old stem	6.28 a	58.00 a	68.34 a	76.00 a
Cutting site	0.00 b	1.63 d	1.00 c	0.92 c
C.V.(%)[1]	51.64	19.60	14.87	3.56

DAT: days after application of treatments. Means followed by the same lowercase letter in the column, comparing the percentage of necrotic area for each treatment, indicate no significant difference by the Tukey test (P<0.05). [1] Coefficient of variation.

On the contrary, picloram application in the cut stem of a woody species (*Tectona grandis)* showed translocation via phloem, being efficient in controlling future shoots (Caldeira and Castro, 2012), which shows a difference in systemic herbicides about those with less mobility and support the importance of knowing the behavior of the applied herbicide and the target weed. In the present study, the necrotic region resulting from stem application must be related to chlorophyll in the fleabane stem, which has a photosynthetically active area. The interaction of protoporphyrin IX with oxygen in

presence of light generates singlet oxygen, resulting in lipid peroxidation of membranes and interruption of chlorophyll and heme synthesis (Owen *et al*., 2011). Moreover, products tend to produce hydrogen peroxide and lead to rapid necrosis and wilting, disrupting cell membranes and reducing herbicide translocation (Eubank *et al*., 2013).

Saflufenacil application on the stem located close to the base (old stem) showed a larger necrotic area at 3, 5, and 7 DAT than the other treatments, demonstrating that this region has greater herbicide translocation capacity (Table 4). The biochemical characteristics of saflufenacil, such as pKa 4.4, octanol-water partition coefficient (Kow 2.6), and ion trapping mechanism, provide it with metabolic stability and assist in its translocation in the plant (Kleier *et al*., 1996). When this herbicide is applied to the basal region of *Conyza* spp. plants, these characteristics allow transport via xylem for a longer period before total destruction of plant structures occurs, since damage to vascular tissues occurs more slowly after absorption. Moreover, it is known that photoassimilate distribution occurs from the producing region (source) to the metabolic and/or storage regions (sink) (Taiz and Zeiger, 2017). These factors may explain the larger necrotic area when saflufenacil was applied in the region close to the base, considered a source region.

CONCLUSION

Saflufenacil is efficient in the control of *Conyza* spp. having a higher action in leafless and cutting plants. The saflufenacil absorption is carried out by the stem in *Conyza* spp. plants. The leaves showed low absorption and translocation from the located application of saflufenacil and this herbicide translocated from the stem to the leaves.

ACKNOWLEDGMENTS

The authors thank to Federal University of Santa Maria for the structural support.

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Revista Facultad Nacional de Agronomía

Selectivity index of indaziflam to sugarcane cv. IACSP95-5000 in two soil textures

UNIVERSIDAD NACIONAL DE COLOMBIA

Índice de selectividad de indaziflam para caña de azúcar cv. IACSP95-5000 en dos texturas de suelo

https://doi.org/10.15446/rfnam.v74n2.92423

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ABSTRACT

Selectivity index is a way of assessing the discrimination of herbicide to a given crop by observing its effects on the crop and the weeds. The aim was to obtain the selectivity index of indaziflam herbicide to sugarcane cultivar IACSP95-5000 as a function of five weed species in two soils textures. The experiment was carried out in a greenhouse at Piracicaba, São Paulo, Brazil. The treatments consisted of indaziflam doses (0; 12.5; 25; 50; 100; 200; 400; 800 and 1,600 g of the active ingredient (ai) ha^{-1}), applied in pre-emergence of the sugarcane and of the weeds *Urochloa decumbens, Urochloa plantaginea, Digitaria horizontalis, Panicum maximum and Rottboellia cochinchinensis*. In sandy loam soil, a 100% control for all weeds was provided at 25 g ai ha^{-1}. In clay soil, for *D. horizontalis* the 90% reduction in total dry mass (ED_{90}) was obtained at 25 g ai ha^{-1}, for *R. cochinchinensis* at 193 g ai ha^{-1}, for *U. plantaginea* at 152 g ai ha^{-1}, for *P. maximum* at 124 g ai ha^{-1}, and for *U. decumbens* at 94 g ai ha^{-1}. Indaziflam was selective to IACSP95-5000 in both soils, with 10% of reduction in dry mass (ED_{10}) at 137 g ai ha^{-1} for soil with a sandy loam texture and 353 g ai ha^{-1} for clay soil. The selectivity index was higher than 1 for all weeds in clay soil. It was not possible to obtain the selectivity index for sandy loam soil due to species susceptibility to the herbicide.

Keywords:
Pre-emergence
Saccharum spp.
Urochloa decumbens
Weed control

RESUMEN

El índice de selectividad es una forma de evaluar el efecto selectivo de un herbicida para un cultivo determinado mediante la observación de sus efectos sobre el cultivo y las malezas. El objetivo fue obtener el índice de selectividad del herbicida indaziflam en el cultivo de caña de azúcar IACSP95-5000 en función de cinco malezas en dos texturas de suelo diferentes. El experimento se realizó en un invernadero en Piracicaba, São Paulo, Brasil. Los tratamientos consistieron en dosis de indaziflam (0; 12,5; 25; 50; 100; 200; 400; 800 y 1,600 g ai ha^{-1}), aplicadas en pre-emergencia de la caña de azúcar y de las malezas *Urochloa decumbens*, *Urochloa plantaginea*, *Digitaria horizontalis*, *Panicum maximum* y *Rottboellia cochinchinensis*. En suelo franco arenoso, 25 g de ia ha^{-1} proporcionaron un control del 100% para todas las malezas. En suelo arcilloso, para *D. horizontalis* la reducción del 90% en la masa seca total (ED_{90}) se obtuvo a 25 g ai ha^{-1}, para *R. cochinchinensis* a 193 g ai ha^{-1}, para *U. plantaginea* a 152 g ai ha^{-1}, para *P. maximum* a 124 g ia ha^{-1}, y para *U. decumbens* a 94 g ia ha^{-1}. Indaziflam fue selectivo para IACSP95-5000 caña de azúcar en ambos suelos, con 10% de reducción de masa seca (ED_{10}) a 137 g ia ha^{-1} para suelo con textura franco arenosa y 353 g ia ha^{-1} para suelo arcilloso. El índice de selectividad fue superior a 1 para todas las malezas en suelo arcilloso. No fue posible obtener el índice de selectividad para suelos franco arenosos debido a la susceptibilidad de las malezas al herbicida.

Palabras clave:
Pre-emergencia
Saccharum spp.
Urochloa decumbens
Control de malezas

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Received: February 19, 2021; Accepted: March 26, 2021
Rev. Fac. Nac. Agron. Medellín 74(2): 9531-9539. 2021

ISSN 0304-2847 / e-ISSN 2248-7026

In the sugarcane crop, chemical control of weeds is the most used method (Reis *et al*., 2019), with high use of herbicides, mainly due to the large extension of cultivated areas. For effective chemical control, it is especially important to know the mode of action of the herbicides, the factors involved in the selectivity, and the behavior of the herbicides in the soil (Victoria-Filho and Christoffoleti, 2004).

Herbicide selectivity can be defined as the ability of an herbicide to control weeds without affecting the plants of interest, that is, without reducing crop productivity (Rasmussen *et al*., 2008). It can occur because of the factors related to the characteristics of plants, such as translocation and differential absorption, age of plants, cultivar, differential metabolism, dimensions of seeds or propagation structures, and factors related to the characteristics of herbicides and their modes of application, such as dose, formulation, and selectivity by position (Oliveira Júnior and Inoue, 2011).

In addition to factors related to plants, the selectivity of herbicides also depends on the type of soil, with the textural class associated with the organic matter content being one of the main factors involved in the dynamics of herbicides in the soil (Silva and Silva, 2007; Inoue *et al*., 2009). As a result, the physical, chemical, and biological characteristics of the soil directly influence the sorption of the herbicide, affecting the amount of the molecule available in the solution and consequently the selectivity of herbicides applied in pre-emergence (Karpinski *et al*., 2014).

The weeds that make up the seed bank can also be a factor in determining the selectivity of the herbicide, where plants are difficult to control at higher doses, and this can increase the possibilities of injury in the crop. The evaluation of the toxicity of an herbicide to a specific crop can be carried out by assigning visual notes according to the severity of the injuries, comparing them as treated plants with the control where there is no herbicide application. Nevertheless, in some conditions, reductions in crop yields are observed after herbicide application, even though they do not cause visual injuries (Carvalho *et al*., 2009). Therefore, when evaluating selectivity, in addition to visual symptoms of intoxication, it is important to consider factors related to yield (Monquero *et al*., 2011).

According to Ritz and Streibig (2005) in some cases, injury can be accepted with low damage symptoms or a reduction in production if effective weed control is made. For example, a 10% reduction can be tolerated (ED_{10}), while 90% weed control (ED_{90}) can be considered a satisfactory level of control. Thus, other methods can be adopted to evaluate the selectivity of herbicides, such as the selectivity index (SI). This index can be defined as the ratio between the ED_{10} of the crop and the ED_{90} of the weed. The higher the SI, the more selective the herbicide will be (Wang *et al*., 2018).

Indaziflam is an herbicide belonging to the alkylazine chemical class, using in pre-emergence of liliopsidas and magnoliopsidas weeds (Brosnan *et al*., 2012). This molecule presents mainly selectivity to semi-perennial and perennial crops, having low selective for annual crops (Guerra *et al*., 2013).

For this reason, this study aimed to determine the selectivity of the herbicide indaziflam, calculating the selectivity index for the sugarcane crop (IACSP95-5000 cv.), in the analysis of five species of weed in two textures of soil.

MATERIALS AND METHODS

Experimental design and conditions

The experiment was performed in a greenhouse in the city of Piracicaba, São Paulo, Brazil (22°42'31.09" S, 47°37'41.81" W), without temperature control, under natural condition, with an irrigation of 5 mm day^{-1}, between January 21 to March 19, 2019. Sandy loam and clay soil were used (Table 1), placing in 5 L plastic pots for planting sugarcane and sowing weeds. The soil was collected from an experimental area located in Piracicaba, in places with low weed infestation.

Five weed species, important in the cultivation of sugarcane, were used: *Urochloa decumbens* (Stapf) R.D. Webster, *Urochloa plantaginea* (Link) R.D. Webster, *Digitaria horizontalis* Willd., *Panicum maximum* Jacq. *Rottboellia cochinchinensis* Lour. Clayton. Plants of other species, which came to emerge in the pots, were removed manually. The sugarcane cultivar IACSP95-5000 (SP84-2066 x SP80-185) was used, which is adapted to most growing regions in the Center-South region of Brazil.

Table 1. Results of chemical and physical analysis of soils. Piracicaba, São Paulo, Brazil.

Clay soil									
pH ($CaCl_2$)	**H+Al**	**P**	**O.M.[1]**	**K**	**Ca**	**Mg**	**SB[2]**	**CEC[3]**	**V[4]**
5.3	47.0	12.0	42.0	2.3	55.0	9.0	66.3	113.3	59
Clay		**Silt**			**Sand**			**Texture**	
43.4		16.0			40.6			Clayey	
Sandy loam soil									
pH ($CaCl_2$)	**H+Al**	**P**	**O.M.**	**K**	**Ca**	**Mg**	**SB**	**CEC**	**V**
5.4	28.0	10.0	18.0	1.3	26.0	7.0	34.3	62.3	55
Clay		**Silt**			**Sand**			**Texture**	
20.1		5.8			74.0			Sandy loam	

Units: H+Al, K, Ca, Mg, SB and CEC ($mmol_c\ dm^{-3}$); P (resin) ($mg\ dm^{-3}$); O.M. (calorimetry) ($g\ dm^{-3}$); V, clay, silt, sand (%). [1] organic matter, [2] sum of bases, [3] cation exchange capacity, [4] base saturation.

Weed species were sown on January 22, 2019. Sugarcane was planted using three wheels per pot, with a viable yolk per wheel and a planting depth of 0.08 m. 100 seeds per pot were used for each weed species, covered with a soil layer of approximately 0.02 m. Prior to the sowing of weeds, a germination test was performed, with 38% emergence for *U. decumbens*, 16% for *U. plantaginea*, 36% for *D. horizontalis*, 56% for *P. maximum* and 18% for *R. cochinchinensis*.

The treatments consisted of the application of doses of indaziflam (Allion®, Bayer CropScience Ind. Ltda, Brazil), in the five species of weeds. Doses 0, 0.125, 0.25, 0.50, 1, 2, 4, 8 and 16X were used at a dose of 100 g ai ha^{-1}, equivalent to 0; 12.5; 25; 50; 100; 200; 400; 800 and 1,600 g ai ha^{-1} of the herbicide. A completely randomized design with four replications was used, where each pot was comprised of an experimental unit.

The application of indaziflam occurred in total pre-emergence of weeds and sugarcane one day after sowing and planting. At the time of application, the loamy and sandy soils had a humidity of 14.73 and 23.36%, respectively. For the application, a CO_2 sprayer pressurized with 1 bar equipped with four spray nozzles (XR 11002, TeeJet® Technologies South America, Brazil) was used. With a pressure of 2 bar, 50 cm height from the surface of the pots, and at 1 m s^{-1} speed, reaching an applied range of 50 cm wide per spray nozzle, and a spray volume of 200 L ha^{-1}.

Evaluations and data collection

The assessment of dry mass of the shoot and root was performed at 60 days after application (DAA). To generate the selectivity index, sugarcane plants and weed species were collected, being cut close to the ground and packed in paper bags, and the root system was separated and washed under running-water until complete cleaning and later conditioned in paper bags. The samples were dried in an oven with forced air circulation at 65 °C for 72 h and measured on a scale to two decimal places.

Data analysis

The results were subjected to analysis of variance (ANOVA) to verify the effect of doses of indaziflam ($P<0.05$). The non-linear logistic regression model was adjusted using the DRC package in the R software (Streibig, 1988; Ritz *et al.*, 2015), as shown in Equation 1.

$$Y = \frac{\alpha}{1+\left(\frac{x}{b}\right)^{c}} \qquad (1)$$

Where:

Y=Total dry mass; α=maximum value; *x*=indaziflam dose in g ai ha^{-1}; *b*= resulting dose at 10, 50 and 90% reduction in total dry mass (ED_{10}, ED_{50} e ED_{90}); *c*=curve slope.

The SI calculation for sugarcane as a function of weed species was obtained by Equation 2 and took into account the herbicide dose necessary for a reduction

of 10% in the total dry mass of sugarcane and 90% in the total dry mass of weed species, these doses were obtained by the equation:

$$SI=\frac{ED_{10}(sugarcane)}{ED_{90}(weed)} \quad (2)$$

Where: SI=selectivity index; ED_{10}=dose of herbicide that resulting in a 10% reduction in the total dry mass of sugarcane; ED_{90}=dose of herbicide that resulting in a 90% reduction in the total dry mass of the weed.

RESULTS AND DISCUSSION

In sandy loam soil, as weeds were susceptible to indaziflam, 0.25 of the recommended dose (25 g ai ha^{-1}) has already provided 100% control. As a result, the weed dry mass data did not fit the log-logistic model of three-parameter suggested to estimate the selectivity index. However, *U. decumbens* species, *D. horizontalis* and *P. maximum* were the most controlled weeds by the herbicide in this soil, where at the dose of 12.5 g ai ha^{-1} showed reductions in the dry mass of 90% (Table 2).

Table 2. Selectivity index (SI) and effective dose (ED) of the herbicide indaziflam that cause reductions in dry mass by 10% and 50% for sugarcane and reductions of 50% and 90% in weed species in a sandy-loam soil texture.

Crop/weed	ED_{10} [1]±SE[2]	ED_{50} [3]±SE	ED_{90} [4]±SE	SI[5]
		g ai ha^{-1}		
IACSP95-5000	137.83±81.48	746.01±171.59	-	-
U. decumbens	-	≤12.50	≤12.50	-
U. plantaginea	-	≤12.50	≤25.00	-
D. horizontalis	-	≤12.50	≤12.50	-
P. maximum	-	≤12.50	≤12.50	-
R. cochinchinensis	-	≤12.50	≤25.00	-

[1] dose of herbicide that provides a 10% reduction in total dry mass of crop; [2] standard error of parameters; [3] dose of herbicide that provides a 50% reduction in total dry mass of crop and weed; [4] dose of herbicide that provides a 90% reduction in total dry mass of weed; [5] selectivity index.

Sugarcane IACSP95-5000 in soil with a sandy loam texture showed a 10% reduction in dry mass (ED_{10}) at a dose of 137.83±81.48 g ai ha^{-1} and a 50% reduction in dry mass (ED_{50}) at a dose of 746.01±171.59 g ai ha^{-1} (Table 2). In the clay soil, a higher ED_{10} was exhibited using 353.23±109.37 g ai ha^{-1} and ED_{50} at 671.07±80.62 g ai ha^{-1} (Table 3). Thus, the percentage of reduction in total dry matter (ED_{10}) of this cultivar in soil with a sandy loam texture occurred with a lower dose of the herbicide (Figure 1).

Table 3. Selectivity index (SI) and effective dose (ED) of the herbicide indaziflam that cause reductions in dry mass by 10% and 50% for the cultivar of sugarcane and reductions of 50% and 90% in weed species in clayey soil.

Crop/weed	ED_{10} [1]±SE[2]	ED_{50} [3]±SE	ED_{90} [4]±SE	SI[5]
		g ai ha^{-1}		
IACSP95-5000	353.23±109.37	671.07±80.62	-	-
U. decumbens	-	19.85±3.25	94.02±34.31	3.76
U. plantaginea	-	27.59±7.65	152.00±53.25	2.32
D. horizontalis	-	≤12.50	≤25.00	-
P. maximum	-	10.35±3.78	124.39±52.77	2.84
R. cochinchinensis	-	65.50±10.78	193.02±48.46	1.83

[1] dose of herbicide that provides a 10% reduction in total dry mass of crop; [2] standard error of parameters; [3] dose of herbicide that provides a 50% reduction in total dry mass of crop and weed; [4] dose of herbicide that provides a 90% reduction in total dry mass of weed; [5] selectivity index.

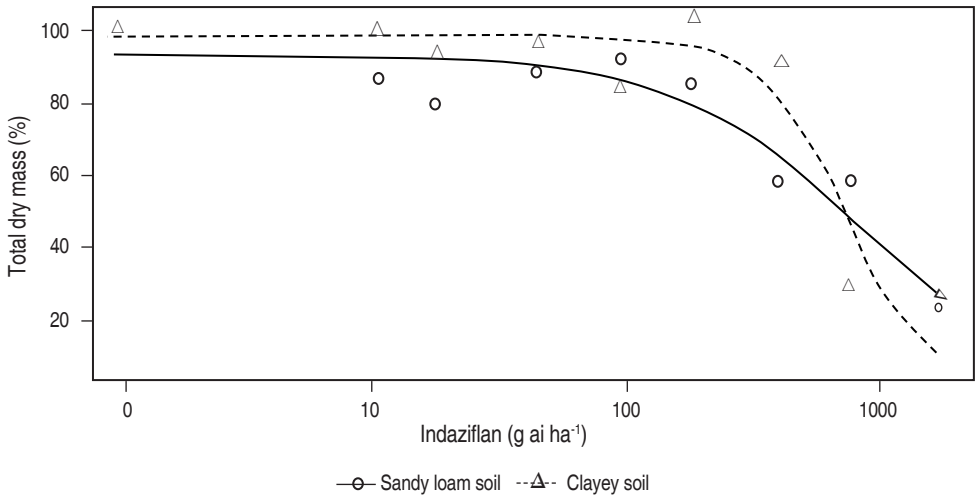

Figure 1. Total dry mass reduction (%) of sugarcane cultivar IACSP95-5000 under indaziflam doses in different soils textures.

In clay soil, the species most susceptible to indaziflam was *D. horizontalis*, where 90% of the species control (ED_{90}) was obtained at a dose of 25 g ai ha^{-1}, with no adjustment possible (Figure 2). The *R. cochinchinensis* was the species less controlled by the herbicide, followed by the species *U. plantaginea*, *P. maximum,* and *U. decumbens*, where the 90% reduction in total dry mass was obtained at doses of 193±50; 152±50; 124±150 and 94±30 g ai ha^{-1}, respectively. The selectivity index was higher due to the species *U. decumbens* being 3.76, followed by the species *P. maximum* with 2.84, *U. plantaginea* with 2.32 and *R. cochinchinensis* with 1.83 (Table 3).

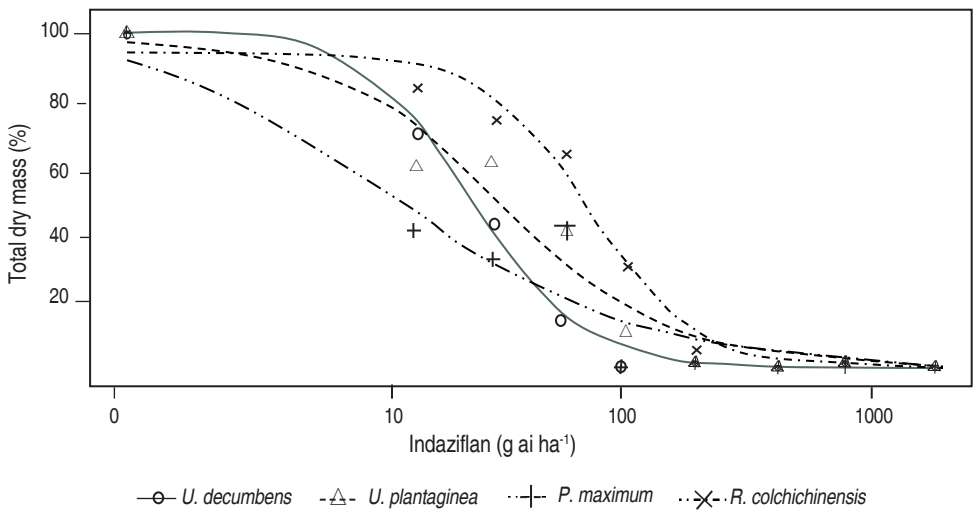

Figure 2. Total dry mass reduction (%) of weeds species under indaziflam doses on clayey soil.

In sandy loam soil, sugarcane root system was the most affected with lower doses of the herbicide indaziflam when compared to its shoot system (Figure 3). The ED_{10} for the root system was obtained with the dose of 95.15±89.59 g ai ha^{-1} and for the shoot it was 162.61±112.12 g ai ha^{-1} (Table 4). The doses of the herbicide for ED_{50} in this same soil were also higher for shoots 938.19±239.04 g ai ha^{-1} and for the root system it was 530.40±147.77 g ai ha^{-1}.

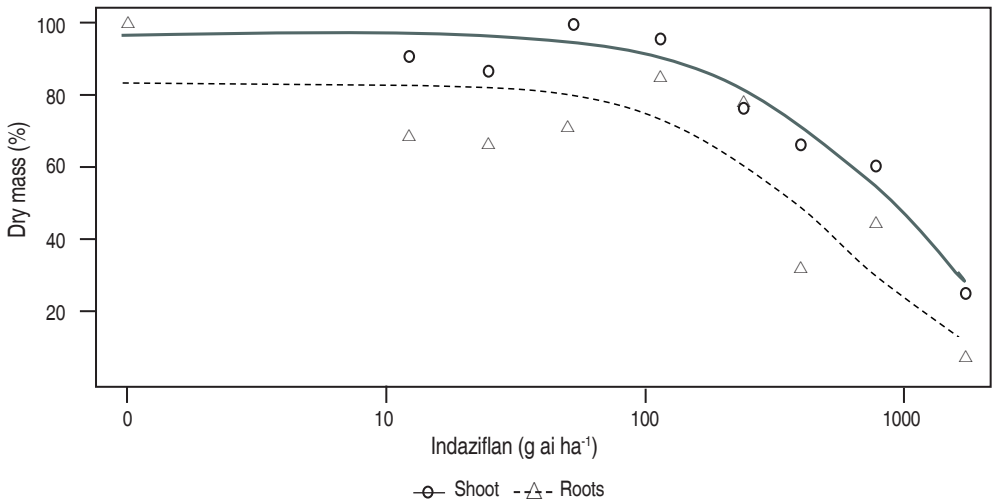

Figure 3. Dry mass reduction (%) from shoot and roots of IACSP95 – 5000 sugarcane cultivar under indaziflam doses on sandy loam soil.

In the clay soil, the root system was more affected with lower doses of the herbicide when compared to the shoot (Figure 4). The ED_{10} was obtained in the doses of 306.93±97.72 g ai ha^{-1} for the root and 361.22±111.81 g ai ha^{-1} for the shoot. However, the doses required for ED_{50} in the root system were higher for this soil, being 837.51±111.49 g ai ha^{-1} for the root and 770.09±71.07 g ai ha^{-1} for the shoot (Table 4).

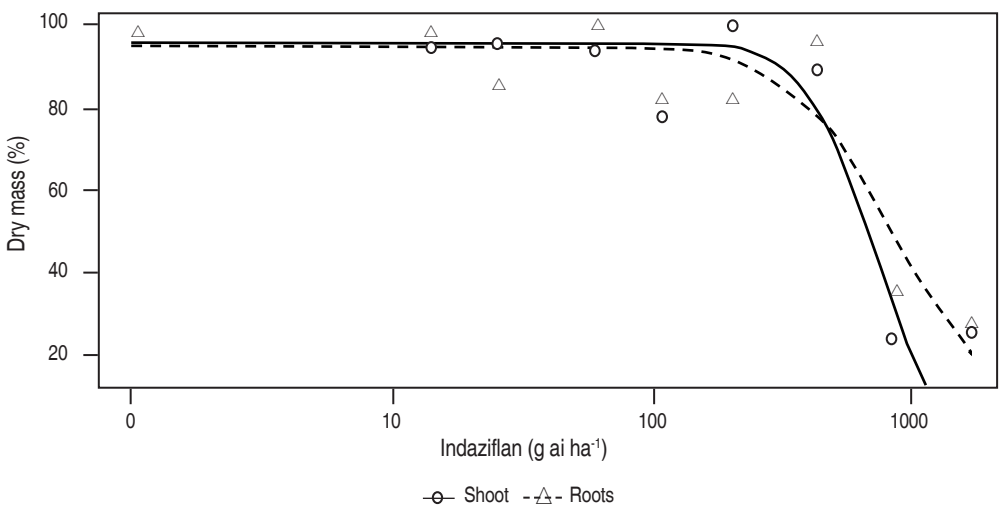

Figure 4. Dry mass reduction of aerial from shoot and roots of IACSP95-5000 sugarcane cultivar under indaziflam doses on clay soil.

The application of indaziflam (0, 30, 60, 90, 120 and 150 g ai ha^{-1}) in pre-emergence of *P. maximum*, *D. horizontalis,* and *R. cochinchinensis*, in three soils with different physical and chemical characteristics, was effective in controlling species. *P. maximum* and *D. horizontalis* were controlled from the dose of 30 g ai ha^{-1}, regardless of the type of soil (Amim *et al*., 2014). The present study corroborates these results, where *D. horizontalis* species was very susceptible to indaziflam regardless of the type of soil, with all species controlled in the lowest doses. However, in clay soil species such as *P. maximum* and *R. cochinchinensis* showed a 90%

Table 4. Indaziflam doses that cause dry mass reductions in dry mass by 10% and 50% of the IACSP95-5000 cultivar depending on the plant part and soil texture.

Soil texture	Plant part	ED_{10} [1]±SE[2]	ED_{50} [3]±SE
		g ai ha^{-1}	
Clayey	Shoot	361.22±111.81	770.09±71.07
	Roots	306.93±97.72	837.51±111.49
Sandy loam	Shoot	162.61±112.12	938.19±239.04
	Roots	95.15±89.59	530.40±147.77

[1] dose of herbicide that provides 10% reduction in shoot and root mass; [2] standard error; [3]dose of herbicide that provides a 50% reduction in the dry mass of shoot and root.

reduction in total dry mass at doses of 124.39±52.77 and 193.02±48.46 g ai ha^{-1} respectively; these doses are higher than those recommended for the herbicide for similar soils (Rodrigues and Almeida, 2018).

Indaziflam was selective for the IACSP95-5000 sugarcane cultivar. In the sandy loam soil, the ED_{10} was lower than the dose required for the same reduction in clay soil. This may have occurred given that indaziflam has a positive correlation to organic matter, due to its high value of the sorption coefficient normalized by organic carbon (Koc<1,000 mg g^{-1}) and a high value of the octanol-water partition coefficient (Kow=2.8), contributing to greater sorption of organic matter and consequently lower availability in the soil solution, impacting on selectivity and weed control (Tompkins, 2010; Alonso *et al*., 2011; Sebastian *et al*., 2017).

These factors may have had an impact on the selectivity and control of the weed species evaluated in this study, since the sandy loam soil has a lower organic matter content (18 mg dm^{-3}) than the clay soil (42 mg dm^{-3}) and consequently, to reach ED_{10}, a lower dose of the herbicide was necessary. Also, in sandy loam soil, indaziflam was effective in controlling weed species with lower doses. Sebastian *et al.,* (2017) evaluated the influence of the physical-chemical properties of the soil on the efficacy of controlling indaziflam on *Kochia scoparia*, and observed that a concentration of 10 to 100 times greater is necessary to promote a 50% growth reduction (ED_{50}) for soil with 16.8% organic matter compared to soil with 0.4%.

Indaziflam application (300 g ai ha^{-1}) did not cause injury to the sugarcane (RB867515 cultivar), in an area with sandy soil (Simões, 2018). However, from the dose of 137.83±81.48 g ai ha^{-1}, reductions of 10% in the total dry mass was observed. In the present study, the selectivity index was not possible to obtain for the sandy loam soil, due to the sensitivity of the species to the herbicide indaziflam in this type of soil. This can be correlated with the physical-chemical characteristics of the soil, being the herbicide more available. It may be considered that indaziflam was very selective in this type of soil, due to the control of the weed species evaluated in the first doses and because it required relatively high doses to cause reductions in the dry mass of the sugarcane cultivar. In clay soil, indaziflam was also considered selective, even with the need for higher doses to control species. A selectivity index value greater than 1 indicates that the herbicide is more selective due to the weed species (Bartley, 1993).

A reduction in dry root mass was observed in the sandy loam soil with lower doses of indaziflam (95.15±89.59 g ai ha^{-1}), when compared to the shoot in this same soil (162.61±112, 12 g ai ha^{-1}). In clay soil, the doses of indaziflam required for a 10% reduction in root dry matter were 306.93±97.72 g ai ha^{-1}. The reduction in biomass and morphology of the root system by indaziflam has also been observed in other studies (Jones *et al*., 2013; Brabham *et al*., 2014; Jones *et al*., 2015; Schneider *et al*., 2015).

Schneider *et al.* (2015) obtained similar results to the present study, using *Cynodon dactylon* plants, in the sand, regardless of the indaziflam dose, an injury was observed, with a root and shoot reduction of 32 and 10% up to 10 cm

depth, respectively. This may be a factor that influenced the results of this study, as the sugarcane stems were planted at 8 cm depth, where the first roots may have come into contact with the herbicide. The authors also observed that the increase in clay content and especially the addition of organic matter, significantly improved the root mass at a dose of 16 g ai ha^{-1} compared to sandy soil without organic matter. However, this same dose caused inhibition in the growth of the roots, without influence on the development of the shoot of the plant in the sandy and clay soils even with organic matter.

The physico-chemical properties of indaziflam suggest that the molecule can be moderately mobile in the soil (solubility in water (Sw)=4.4 mg L^{-1} at pH 4.0) due to the long persistence (half-life time ($T_{1/2}$)>150 days) associated with low solubility in water (Sw=2.2 mg L^{-1} at pH 7.0 to 9.0) and moderate sorption (sorption coefficient (Kd)=4.9 to 27.4 g mL^{-1}) (Tompkins, 2010; Alonso *et al.*, 2011). Nevertheless, as the sandy loam soil has a lower content of organic matter (18 mg dm^{-3}) compared to the clay one (42 mg dm^{-3}), and the herbicide has a great affinity to organic matter (Koc<1,000 mg g^{-1}), and with the presence of water during the entire conduction of the experiment, indaziflam may have had more mobility in the sandy loam soil, causing greater reductions in the root system of sugarcane in this soil.

CONCLUSIONS

In sandy loam soil, the indaziflam dose of 25 g ai ha^{-1} provided 100% control for all weeds. In clay soil, for *D. horizontalis* the ED_{90} was obtained at 25 g ai ha^{-1}, for *R. cochinchinensis* at 193 g ai ha^{-1}, for *U. plantaginea* at 152 g ai ha^{-1}, for *P. maximum* at 124 g ai ha^{-1}, and for *U. decumbens* at 94 g ai ha^{-1}. Indaziflam was selective to IACSP95-5000 sugarcane cultivar in both soils, with ED_{10} at 137 g ai ha^{-1} for soil with a sandy loam texture and 353 g ai ha^{-1} for clay soil. The selectivity index was higher than 1 for all weeds in clay soil. It was not possible to obtain the selectivity index for sandy loam soil due to species susceptibility to the herbicide.

ACKNOWLEDGMENTS

This study is a derivation of the master thesis of the first author, financed by *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (Capes, BR).

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Revista Facultad Nacional de Agronomía

UNIVERSIDAD NACIONAL DE COLOMBIA

Weed interference capacity on soybean yield

Capacidad de interferencia de las malezas en la productividad de la soya

https://doi.org/10.15446/rfnam.v74n2.89705

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ABSTRACT

Among biological factors, weeds are the most important limiting factor for crop yields, as well as increasing production costs. The aim was to determine the influence of control and coexistence of weed community on soybean crop yield and to define the period before interference, the critical period of interference prevention and the total period of interference prevention, with the comparative use of chemical and mechanical methods for weed eradication. The study was conducted in an experimental field in the 2018/2019 harvest. A randomized block with four replications was implemented as experimental design, using two methods for control. The evaluated periods were 0-10, 0-20, 0-30, 0-40, 0-50, 0-60 and 130 days after crop emergence. It was possible to observe that the use of the chemical method generated a higher yield compared to mechanical method. The period before the interference in both chemical and mechanical management was similar, approaching 20 days after crop emergence. The critical period of interference prevention was between 20-50 and 40.5 days after crop emergence in chemical and mechanical methods, respectively. The total period of interference prevention was extended to 50 and 40.5 days after crop emergence in chemical and mechanical methods, respectively. The reduction in productivity due to weed interference was 1639 kg ha^{-1} (55%) and 947 kg ha^{-1} (34.6%) in chemical and mechanical methods, respectively.

Keywords:
Coexistence
Control
Glycine max
Productivity
Suppression

RESUMEN

Entre los factores biológicos, la maleza es el factor restrictivo más importante del rendimiento de los cultivos, además de aumentar los costos de producción. El objetivo fue determinar la influencia del control y la coexistencia de la comunidad de malezas en el rendimiento del cultivo de soya y definir el período anterior a la interferencia, el período crítico de prevención de las interferencias y el período total de prevención de las interferencias, con el uso comparativo de métodos químicos y mecánicos para la erradicación de las malezas. El estudio se realizó en un campo experimental en la cosecha de 2018/2019. Se implementó un diseño experimental de bloque aleatorio con cuatro réplicas, utilizando dos métodos para el control. Los períodos evaluados fueron 0-10, 0-20, 0-30, 0-40, 0-50, 0-60 y 130 días después de la aparición de las plantas. Se pudo observar que el uso del método químico generó un mayor rendimiento en comparación con el método mecánico. El período anterior a la interferencia tanto en el manejo químico como mecánico fue similar, acercándose a los 20 días después de la aparición de las plantas. El período crítico de prevención de la interferencia fue entre 20-50 y 40,5 días después de la aparición de las plantas en los métodos químico y mecánico, respectivamente. El período total de prevención de la interferencia se extendió hasta los 50 y 40,5 días después de la aparición de las plantas según método químico y mecánico, respectivamente. La reducción de la productividad debida a la interferencia de las malezas fue de 1639 kg ha^{-1} para el método químico (55%) y 947 kg ha^{-1} (34,6%) para el mecánico.

Palabras clave:
Coexistencia
Control
Glycine max
Productividad
Supresión

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Received: January 22, 2021; Accepted: April 01, 2021
Rev. Fac. Nac. Agron. Medellín 74(2): 9541-9547. 2021

ISSN 0304-2847 / e-ISSN 2248-7026

The quality of the soybean crop can be affected by several factors, being the competition of the crop with weeds one of the most important elements when a higher yield is the target. The interference in soybean crop performance by weeds can result in losses of up to 90%, if effective control methods are not used (Silva *et al.,* 2009; Almarie, 2017). The dispute for the same resources, essential for the growth and development of weed and the crop of interest, when limited in quantity to satisfy the individual requirements present in the environment causes interference, resulting in productivity reduction of the crop of interest, in the final quality of the harvested product and impacting the economic result of the crop (Balbinot *et al.,* 2016).

According to Vargas *et. al.,* (2016), by considering only the state of Rio Grande do Sul, the presence of areas with resistant weeds and the additional cost of using alternative herbicides for their control, combined with the production losses due to competition between crops and weeds estimated at 10 to 20% of production, exceed $1 billion in each crop. In the national scenario, the total cost of weed resistance to herbicides with an average productive interference in soybean crop of 5% may exceed $2 billion each year (Adegas *et. al.,* 2017).

According to Radosevich *et al.* (2007), the competition between weeds and the crop of interest is divided into three periods, period before to interference (PBI), critical period of interference (CPIP) and total period of interference prevention (TPIP). PBI is the period in which weeds occurrence does not cause yield losses, starting at the emergence of the crop and extending to the beginning of the CPIP, which is the most relevant phase of the competition because it comprises the most critical period in which the crop is more susceptible to the damage caused by the presence of plants in the area and the coexistence between the two species causes yield losses and grain quality in greater evidence (Radosevich *et al.,* 2007; Agostinetto *et al.,* 2014; Zandoná *et al.,* 2018). The CPIP starts at the end of the PBI and extends until not causing any more significant damage to the crop (Radosevich *et al.,* 2007). TPIP covers the two periods aforementioned, encompassing the sum of both.

To determine each period, to evaluate the effect of different times of weed management on phytosociological indices and to determine the periods of weed interference on crop yield, controlling the intensity of weed interference on the crops of interest, is extremely important regarding strategies for weed management.

Thus, the aim of this study was to determine the influence of control and coexistence of the infesting weed community on soybean crop productivity and to define the PBI, the CPIP and the TPIP with the comparative use of chemical and manual methods for weed eradication, as well as the loss of productivity caused by intraspecific competition.

MATERIALS AND METHODS

The research was conducted in the experimental area of the Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Sul during 2018/2019, with 705 m of altitude. The soil of the experimental area is classified as typical dystrophic Red Nitosol (Streck *et al.,* 2018). The climate is hot and temperate (Cfa in the Köeppen and Geiger classification) with average annual rainfall of 1971 mm and average annual temperature of 17.8 °C.

The design of the experiment was in randomized blocks. The presence (coexistence) and absence (control) of weeds in soybean crop were evaluated for seven periods of weed coexistence with the crop: 0-10, 0-20, 0-30, 0-40, 0-50, 0-60 and 130 days after crop emergence (DAE). During these periods, the coexistence management consisted of keeping soybean in the presence of weeds and then controlled until the end of the cycle. During the control periods, the crop remained weed-free in the same periods.

The experiment area was planted with black oat during the previous season. The soybean cultivar used was BMX Lança IPRO 58i60 RSF, which presents an indeterminate growth habit and maturation group 5.8, with average plant size and undetermined growth habit, requiring high soil fertility and presenting a total development cycle of 142 days in the region. The local management of the cultivated straw was a no-tillage system with fertilization in the sowing line using the formulation 20-30-20 N-P-K, at a dose of 380 kg ha^{-1}, as being adjusted according to the soil analysis and the crop productivity expectations.

The experimental area was managed under the consolidated no-tillage system, where the previous used crop was black oat for the purpose of soil coverage. The

management was carried out 30 days before soybean sowing, using a sequential application of herbicides Cletodim (144 g ai (active ingredient) ha^{-1})+Glyphosate (854.4 g ai ha^{-1}) and in pre-sowing the herbicide Paraquat (400 g ai ha^{-1}).

The counting and identification of weed population were performed in the interval of 10 days, coinciding with the periods of coexistence and interference. Weed control was based on the use of a chemical method (herbicides), since it is the most used, and also, the use of the mechanical method, pulling out weed present in the sowing line and weeding between the rows of the crop.

For the chemical control of weeds, chlorimuron or Cletodim associated with glyphosate were applied, according to the predominant population at the time of application, performing an association of glyphosate herbicides (854.4 g ai ha^{-1}), and Cletodim (108 g ai ha^{-1}) in the predominance of monocotyledon weeds, or the combination of glyphosate herbicides (854.4 g ai ha^{-1}) and clorimuron (20 g ai ha^{-1}) when dicotyledons were predominated.

In order to determine the PBI, a regression equation with three parameters was used, according to Velini *et al.* (1997):

$$Y = a / [1+ (x / x_0)^b] \quad (1)$$

where: Y=grain yield; a=maximum yield obtained in the clean control; x=number of days after crop emergence; x_0=number of days in which 50% of maximum yield reduction occurred; and b=curve slope.

Regarding the data referring to the control period, the following equation of four parameters was used:

$$Y = y_0 + c / [1 + (x/x_0)^b] \quad (2)$$

where: y_0=minimum yield obtained in the infested treatment; c=difference estimated by the model between the maximum yield in the control treatment (without weeds) and the minimum yield in the infested treatment. The other parameters are similar to those of the previous equation. To determine the critical period of interference prevention, the value of the coexistence period was subtracted from the total period of interference.

PBI was estimated considering a 5% reduction in crop maximum productivity in each of the management, being defined as the average cost to control the weed community present (Silva *et al.,* 2015; Silva *et al.,* 2016; Agostinetto *et al.,* 2020).

The harvesting process was carried out in a useful area of 4.05 m², manually. After tracking the material, impurities were removed, moisture determination and weighing of each sample was performed. The data were compared by Tukey's test at 5% significance using the ASSISTAT statistical program (v.7.7). The regression curves were constructed by Sigmaplot software (v.12.5).

RESULTS AND DISCUSSION

The weed-infesting community covered several species, of which stand out *Conyza* spp. (horseweed), *Bidens pilosa* (black-jack), *Euphorbia heterophylla* (milkweed), *Raphanus sativus* (radish), *Lolium multiflorum* (ryegrass), *Amaranthus hybridus* (green amaranth), *Digitaria horizontalis* (crabgrass), *Urochloa plantaginea* (alexandergrass), *Ipomoea purpurea* (morning-glory), and *Eleusine indica* (indian goosegrass).

The presence of these weeds, predominantly those of the Magnoliopsida family in the considered area, is related to the record of the management of the area. Heterogeneity of weeds causes different flows during the development of the crop of interest, which makes weed management more complex and requires a management of a wide control spectrum during a considerable period of time. Furthermore, species of the Magnoliopsida family can be considered to be potentially more harmful to the soybean cultivation, given that they present characteristic cycles, root system and nutritional needs similar to the crop (Rizzardi *et al.,* 2004).

The use of chemical management for weed control was selective, not causing phytotoxicity to the crop and thus not affecting productivity, as well as allowing a satisfactory weed control. This fact is explained by the use of herbicides at 10-day intervals, ensuring weed control in its initial stages, where they have high sensitivity (Oliveira and Brighenti, 2018), as well as in early stages of the crop, allowing the herbicide to reach effectively the weed (Souza *et al,* 2018).

By considering the evaluated data, there was no interaction between the factors. There was interaction between chemical and mechanical management with the 7 periods evaluated at 5% level of significance. When the singular factors were evaluated, a statistical difference was observed between the initially clean period and initially dirty period at 1% level of significance.

Regarding the period of coexistence of the crop with weeds (coexistence), it was possible to observe that even when sowing of the crop without the presence of weeds, the propagules developed very early and as soon as the competition began, as well as they interfered in the productive potential of the crop (Figure 1). However, when considering the cost of herbicide use and the use of machines corresponding to 5% of productivity to define the period prior to interference, there are no productive losses of the crop due to the effect of coexistence with weeds until 20 DAE.

Thus, it is possible to affirm that when the crop and weeds are in early stages of development, there is no harmful competition for these individuals (Zandoná *et al.,* 2018), which can be considered the period before interference. However, monitoring the crop since the emergence, through weed control in early stages, ensures better control of the infesting population (Agostinetto *et al.,* 2009), considering that the increase of density of these plants, especially those of that develop at the beginning of the crop cycle, accentuate the competition for water, light and nutrients (Souza *et al.,* 2019).

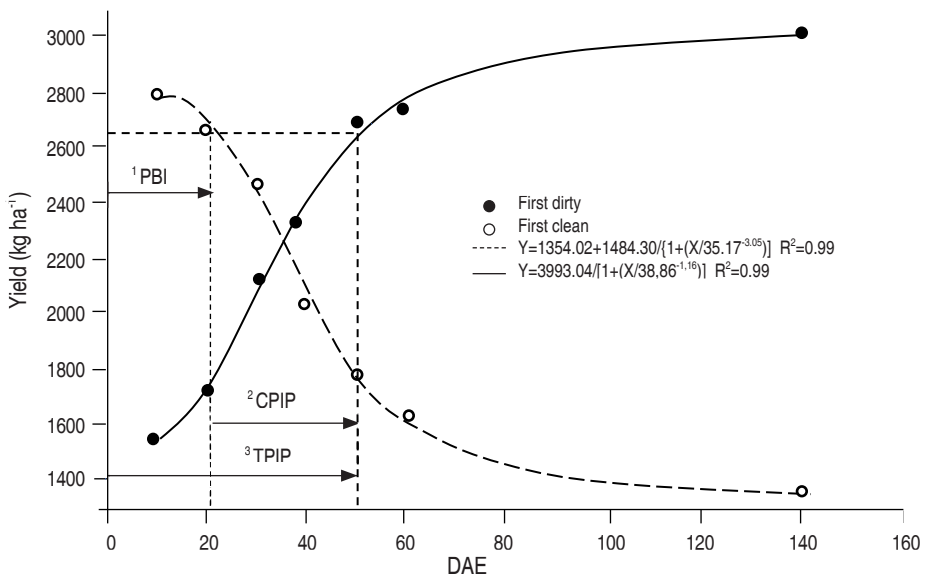

Figure 1. Period of coexistence and interference of soybean crop with weeds through chemical management. Sertão-RS/Brazil, 2019. PPI: period before interference; CPIP: critical period of interference; TPIP: total period of interference prevention; DAE: days after crop emergence.

The critical period of interference extended from 20 to 50 days. During this period, there was a reduction in productivity higher than 33% where no weed management was used, highlighting the loss of productive potential that weed interference causes in soybean crop. However, the study conducted by Nonemacher *et al.,* (2017) showed that the application of post-emergent herbicides when the crop was in the V3 stage (three fully developed trifoils) ensured that there was no significant interference of weeds in productivity, reinforcing the relevance of the initial period of crop development.

Thus, it is important to emphasize that each weed species as well as each cultivar have unique characteristics in relation to the interference and/or competition capacity. According to Danilussi *et al.* (2019), a plant m^{-2} of *Digitaria insularis* has the capacity to reduce 22.98%

of crop productivity. Moreover, according to Zandoná *et al.* (2018), productivity losses due to weed interference reached 93.7%, which emphasizes that the evaluated crop shows good competitive characteristics.

When weed control was performed mechanically (Figure 2), a pulling out weed was implemented in the sowing line and weeding between the rows of the crop. At each moment of mechanical management, a higher weed population was observed because of the higher regrowth rate in the periods of higher soil moisture. In contrast, the chemical method was more effective in the control of weed population. The use of the mechanical method when performed in periods of higher soil moisture, allows a higher rate of regrowth compared to the chemical method and propitiates the propagation of species that reproduce vegetatively (Jakelaitis *et al.,* 2003).

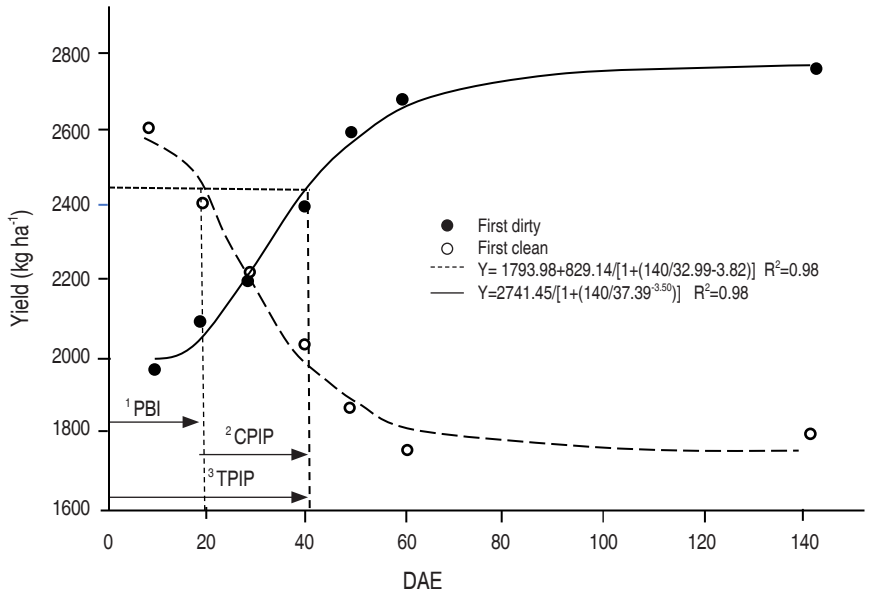

Figure 2. Period of coexistence and interference of soybean crop with weeds through mechanical management. Sertão-RS/Brazil, 2019. PBI: period before interference; CPIP: critical period of interference; TPIP: total period of interference prevention; DAE: days after crop emergence

When comparing the maximum crop yield between Figure 1 and 2, it is possible to observe that there was a lower productivity (8.4%) when the mechanical management of weeds was used. This difference may be related to the turnover of the surface soil layer, which can cause mechanical damage to roots of the crop, affecting productivity directly (Stall and Dusky, 2006). Besides, the rate of weed emergence increases, since the no-tillage system, by not turning over the soil, allows to accumulate most of the propagules of these species at 0-5 cm layer (Scherner *et al.,* 2016). Thus, the turnover of this soil layer exposes the seeds to light and variations in temperature, moisture and oxygenation and stimulates the emergence of dormant viable propagules (Vivian *et al.,* 2008).

During crop development, when reaching a size that closes the space between the sowing lines, a shadow on the soil is produced. According to Oliveira and Briguenti, (2018), this condition hinders the emergence of new weed flows reducing the amount of radiation incident in the most low-level populations (Maciel *et al.,* 2004). This condition prevents the need for weed management, both for reducing the emergency flow and for the inability of this population to interfere in crop productivity. This period takes place after the total period of interference until the physiological maturation of the crop.

The use of weed management, regardless of the method used, ensures a satisfactory crop productivity. The loss of productivity due to the competition of crops with weeds without chemical or mechanical management was 1639 kg ha^{-1} (55%) and 947 kg ha^{-1} (34.6%), respectively. These data show the importance of using efficient management, which allows the development of

the crop without competition for space, water, sunlight and nutrients.

It is also noteworthy that the competition may vary according to the intrinsic morphological characteristics of each cultivar, depending on the planting season and density, the initial development, phytomass production, leaf area index, size and architecture of the aerial parts and the adaptability to the growing region (Oliveira and Briguenti, 2018; Brighenti and Oliveira, 2011). In addition to direct interference in the productive parameters of the crop, other problems observed are interference in the harvest, contamination of the harvested product with seeds and other plant parts, an increase in the moisture of the harvested product, impairing its processing and reducing its commercial value (Brighenti and Oliveira, 2011).

Weed management is essential to ensure good crop yield, mainly managing the propagation of emerging weeds until the gap between the sowing lines is closed. Therefore, chemical control is the most efficient method evaluated in weed control, reducing the impacts of interspecific interferences. It is essential to carry out good crop management and the use of rotation, in addition to using both methods interchangeably to ensure the economic success of the activity and the sustainability of the agricultural system.

CONCLUSION

The use of the chemical method ensures a higher crop productivity compared to the mechanical method. The period before the interference in both chemical and mechanical managements was similar, approaching 20 DAE. The critical period of interference prevention was from 20 DAE to 50 and 40.5 DAE in chemical and mechanical methods, respectively, in a total of 30 and 20.5 days. The reduction in productivity due to weed interference was 55 and 34.6% in chemical and mechanical methods, respectively.

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Revista Facultad Nacional de Agronomía

Effect of phytobiotic - Germivit on the functional state of cattle

UNIVERSIDAD NACIONAL DE COLOMBIA

Efecto del fitobiótico - Germivit sobre el estado funcional del ganado

https://doi.org/10.15446/rfnam.v74n2.85369

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ABSTRACT

Plant-based feed additives, also known as phytobiotics, show great promise to compensate deficiencies of important biologically active substances in the diet. Therefore, this study aimed to evaluate the effect of Germivit on the feed supplement of cattle of different ages. In the first experiment, four groups of 9-month-old Simmental calves (10 animals in each group) were fed with Germivit at 0.0, 0.5, 0.7, and 0.9 g kg^{-1} live body weight, respectively. In the second experiment, three groups of pregnant cows (10 animals in each group) received Germivit at 0.0, 0.25, and 0.50 g kg^{-1} live body weight, respectively. The morphological composition of the collected blood was studied using a PCE-90vet automatic hematology analyzer. The blood biochemical composition was studied using a Stat Fax 1904 biochemical analyzer, and the immunological status of the animals was evaluated using generally accepted methods in veterinary medicine: immunocompetent cells by spontaneous rosette formation; immunoglobulins by the radial immunodiffusion method; phagocytosis by using *S. aureus* culture; serum lysozyme activity by the photoelectrocolorimetric method; and bactericidal activity of serum by determining the degree of growth inhibition of the mixture of daily culture of *E. coli* in a nutritive culture broth. Germivit contributed to the improvement of the biochemical and immunological parameters in the calves. In the cows, an increase in the morphological parameters of blood was observed, their immune status improved, and their calves were born with high rates of natural resistance and health. Germivit had a positive effect on the functional state of cattle.

Keywords:
Cattle
Feed supplement
Germivit
Immunity
Metabolism

RESUMEN

Los aditivos de origen vegetal para alimento animal, también conocidos como fitobióticos, son muy prometedores para compensar una deficiencia de sustancias biológicamente activas importantes en la dieta. Este estudio tuvo como objetivo evaluar el efecto del Germivit como suplemento alimenticio del ganado de diferentes edades. En el primer experimento, cuatro grupos de terneros (10 animales en cada grupo) Simmental de 9 meses de edad fueron alimentados con Germivit a una dosis de 0,0; 0,5; 0,7 y 0,9 kg^{-1} de peso corporal vivo, respectivamente. En el segundo experimento, tres grupos de vacas preñadas (10 animales en cada grupo) recibieron Germivit a una dosis de 0,0; 0,25 y 0,50 g kg^{-1} de peso corporal vivo, respectivamente. La composición morfológica de la sangre recogida se estudió utilizando un analizador hematológico automático PCE-90vet. La composición bioquímica de la sangre se estudió utilizando un analizador bioquímico Stat Fax 1904, y el estado inmune de los animales se evaluó utilizando métodos generalmente aceptados en veterinaria: células inmunocompetentes por formación espontánea de rosetas; inmunoglobulinas por el método de inmunodifusión radial; fagocitosis usando cultivo de *S. aureus*; actividad de la lisozima sérica por el método fotoelectrocolorimétrico; y actividad bactericida del suero determinando el grado de inhibición del crecimiento de la mezcla de cultivo diario de *E. coli* en un medio nutritivo líquido. Germivit contribuyó a una mejora de los parámetros bioquímicos e inmunológicos en los terneros. En las vacas se observó un aumento de los parámetros morfológicos de la sangre, mejoró su estado inmunológico y sus terneros nacieron con altos índices de resistencia natural y salud. Germivit tuvo un efecto positivo sobre el estado funcional del ganado.

Palabras clave:
Ganado
Suplemento alimenticio
Germivit
Inmunidad
Metabolismo

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Received: July 9, 2020; Accepted: January 5, 2021
Rev. Fac. Nac. Agron. Medellín 74(2): 9549-9561. 2021

ISSN 0304-2847 / e-ISSN 2248-7026

At the animal husbandry stage of development, special attention should be paid to the feed in order to obtain the maximum genetic potential of animal productivity. The most antimicrobial agents by their nature can be attributed to xenobiotics in relation to the body of the animal. Depending on the dose, they can exhibit toxic effects, have an immunosuppressive effect, disrupt metabolic processes, promote intestinal dysbiosis, and accumulate and pollute raw materials in animal products. This makes necessary to find preparations and feed additives devoid of these negative properties and introduce them into the practice of feeding productive animals (Trukhachev *et al.*, 2015).

The attention of researchers and practitioners has recently turned to plant-based feed additives, also known as phytobiotics, which have diverse positive effects on the animal body. Plants contain a complex of biologically active substances that have low toxicity and act comprehensively. They also offer economic benefits, particularly in terms of the availability and low prices of plant materials as well as the relatively uncomplicated technology for the preparation of phytobiotics (Franciosini *et al.*, 2016; Mohiti-Asli and Ghanaatparast-Rashti, 2017). Numerous scientific studies have shown the positive effects of phytobiotics on the productivity and quality of livestock while reducing the cost of feed for unit of production. These positive effects are achieved due to the high level of vitamins, minerals, flavonoids, essential oils, phytoncides, polysaccharides, tannins, among others, in many plants (Castillo-López *et al.*, 2017; Mohammadi Gheisar and Kim, 2018; Oguey and Wall, 2016; Windisch *et al.*, 2008).

The biologically active substances of plants which have immuno-stimulating activity, are able to suppress pathogenic microbiota, have antioxidant effect, and improve the digestibility of feed nutrients (Borda-Molina *et al.*, 2018; Stanley *et al.*, 2014). The ability of phytobiotics to improve the chemical composition and increase the biological value of meat has also been established (Ramiah *et al.*, 2014). Furthermore, herbal preparations, which often contain probiotics, have a positive effect on the microbiota of the gastrointestinal tract of animals (Currò *et al.*, 2017; Drouillard, 2018; Samii *et al.*, 2016; Valero *et al.*, 2014). The inclusion of phytobiotics in the diet of poultry, cows, young cattle, and sheep has shown to increase their productivity (Al-Yasiry *et al.*, 2017; Brogna *et al.*, 2014; Kumar *et al.*, 2014; Samii *et al.*, 2016; Tosi *et al.*, 2013; Wall and Bravo, 2016), and herbal feed additives can serve as a worthy substitute for synthetic drugs, especially antibiotics (Friedman *et al.*, 2002). When choosing feed additives, preference should be given to drugs and biologically active substances that do not pollute the environment, do not accumulate in the animal's body, are capable of being rapidly metabolized, improve the metabolism, and have a positive effect on animal productivity and livestock production quality.

Germivit produced by the company "Pink-Lotus", is a preparation obtained from wheat germ that takes the form of a beige powder. The product contains minerals (potassium, calcium, magnesium, sodium, phosphorus, iron, copper, manganese, zinc), vitamins (B1, B2, B5, B3, B6, B12, E, A, D), amino acids (alanine, arginine, aspartic acid, valine, histidine, glycine, leucine, isoleucine, lysine, methionine, proline, serine, tyrosine, threonine, phenylalanine, cysteine), and polyunsaturated fatty acids (arachidonic, behenic, linoleic, linolenic, myristic, oleic, palmitic, erucic) (Pink-Lotus, 2020). The latter are the precursors of prostaglandins, which take part in the neurohumoral regulation of the tissues and systems of the body. Germivit has antitoxic, antioxidant, and hepatoprotective effects, has immuno-stimulating properties, and has positive effects on the reproductive function of animals. Furthermore, experiments on newborn calves, pigs, and poultry have shown a high therapeutic and prophylactic activity of Germivit in animal diseases (Donnik *et al.*, 2010).

The phytobiotic Germivit meets several aspects of a healthy animal diet. However, the effect of this food supplement on the bodies of cows and calves has not been studied. Therefore, this study aimed to examine the effect of the Germivit feed supplement on cattle of different ages.

MATERIALS AND METHODS

This research consisted of Simmental breed cattle, which were bred in farm conditions of the Orenburg region of the Russian Federation. Animal care and experimental studies were performed in accordance with the instructions and recommendations of Russian

Regulations, 1987 (Order No. 755 on 12.08.1977 the USSR Ministry of Health) and "The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, D.C. 1996)". When performing the research, efforts were made to minimize the suffering of the animals and to reduce the number of samples used.

Two experiments were carried out; in the first one, the effect of different doses of Germivit on the bodies of calves was studied. For this purpose, four groups of 9-month-old calves were formed: three experimental groups and one control group – each group contained 10 animals. The animals of the first experimental group were fed from the 9 months of age with Germivit once a day at a dose of 0.5 g kg^{-1} live weight, with the morning feeding along with concentrated food. For the animals of the second experimental group, the dosage was increased up to 0.7 g kg^{-1}, and for the third experimental group, it was 0.9 g kg^{-1}. The animals of the control group did not receive Germivit. The experiment continued until the animals were 18 months of age. Blood samples were taken from the jugular vein of 9-, 15-, and 18-month calves to assess the effect of Germivit on their body.

The second experiment consisted of evaluating the effect of Germivit on the body of cows 4-5 years-old two months before calving, as well as its effect on the newborn calves. For this, two experimental groups and one control group with 10 animals each were formed. The cows of the first experimental group for two months daily received Germivit once a day at a dose of 0.25 g kg^{-1} of live weight, while for the animals of the second experimental group, the dose was increased to 0.50 g kg^{-1}. The Germivit was provided every day in the morning with concentrated feed for two months. The cows from the control group did not receive any Germivit. Blood sampling was performed on the cows at 60, 30, and 10 days before calving and 7 days after giving birth. Blood was also taken from the calves obtained from the cows of the experimental and control groups at the ages of 1 day and 1 month. In addition, any case of gastrointestinal pathology in the calves and any calf mortalities over 30 days were recorded.

The morphological composition of the collected blood was studied using a PCE-90vet automatic hematology analyzer (USA), the blood biochemical composition was studied using a Stat Fax 1904 biochemical analyzer (USA), and the animals' immune status was evaluated using methods generally accepted in veterinary medicine: immunocompetent cells by spontaneous rosette formation; immunoglobulins by the method of radial immunodiffusion; phagocytosis by using a *S. aureus* culture; serum lysozyme activity by the photoelectrocolorimetric method; and bactericidal activity of serum by determining the degree of growth inhibition of the mixture of daily culture of *E. coli* in a liquid nutrient medium (Shakhov, 2015). Statistical processing of the obtained data was carried out using the SPSS Statistics software package. During static data processing, the mean, the standard deviation and the Student's t-test were calculated.

RESULTS AND DISCUSSION

First Experiment

The biochemical composition of the blood in the calves is shown in Table 1.

The inclusion of Germivit in the diet of calves contributed to a significant increase in the amount of total protein in the blood of the animals. At the age of 15 months, the young cattle of the first experimental group had a higher total protein content in the blood serum (1.64% (P<0.01)) than the animals from the control group. For the animals of the second experimental group, it was 2.14% (P<0.01) higher, and of the third experimental group, it was 2.05% (P<0.01) higher. By the end of the experiment, i.e., at 18 months of age, the animals of the test groups had retained an advantage in relation to the amount of total protein in the blood compared to the animals in the control group. For the first experimental group, it was 1.22% (P<0.05); for the second one, it was 2.00% (P<0.001), and for the third experimental group, it was 2.05% (P<0.001).

Glucose is the main source of energy for the cells of the body. When assessing the carbohydrate metabolism in the calves, it was found that feeding Germivit contributed to higher levels of blood glucose at the age of 15 months, with 3.00 mmol L^{-1} in the control group and 3.11 mmol L^{-1} in the second experimental group an increase of 3.67%. By the age of 18 months, the maximum values of glucose were recorded in animals of the third experimental group, which had 4.23% (P<0.05)

more blood glucose than the control group. The animals of the first and second experimental groups had 3.26% (P<0.05) and 3.91% (P<0.05) higher glucose contents, respectively, than the control calves.

Table 1. Biochemical composition of the blood of the calves fed with Germivit daily.

Indicators	Germivit (g kg^{-1} live weight)	Age (months)		
		9	15	18
Total protein (g L^{-1})	0.0	67.93±0.111	67.75±0.311	67.96±0.101
	0.5	67.96±0.131	68.86±0.172 **	68.79±0.321 *
	0.7	68.02±0.152	69.20±0.163 **	69.32±0.210 ***
	0.9	67.94±0.154	69.14±0.214 **	69.35±0.193 ***
Glucose (mmol L^{-1})	0.0	3.03±0.032	3.00±0.084	3.07±0.022
	0.5	3.01±0.067	3.11±0.011	3.17±0.021 *
	0.7	3.01±0.063	3.11±0.007	3.19±0.011 *
	0.9	3.02±0.055	3.11±0.011	3.20±0.035 *
Total bilirubin (µmol L^{-1})	0.0	3.93±0.122	3.97±0.113	3.96±0.115
	0.5	3.92±0.101	3.93±0.214	3.77±0.024
	0.7	3.91±0.131	3.95±0.180	3.76±0.032
	0.9	3.93±0.096	3.94±0.222	3.75±0.031
Uric acid (µmol L^{-1})	0.0	162.40±1.367	162.90±1.128	164.30±0.251
	0.5	161.40±1.842	162.50±1.325	162.50±0.743
	0.7	161.80±1.771	162.50±1.516	162.80±0.521
	0.9	162.00±1.691	162.70±1.233	162.90±0.556
Cholesterol (mmol L^{-1})	0.0	2.54±0.131	2.63±0.042	2.66±0.042
	0.5	2.56±0.083	2.62±0.032	2.52±0.061 **
	0.7	2.52±0.091	2.64±0.051	2.53±0.021 *
	0.9	2.51±0.082	2.63±0.022	2.54±0.012 *
Triglycerides (mmol L^{-1})	0.0	0.32±0.033	0.35±0.023	0.39±0.009
	0.5	0.31±0.033	0.31±0.007	0.35±0.008 *
	0.7	0.33±0.031	0.30±0.006	0.35±0.011 *
	0.9	0.31±0.031	0.30±0.011 *	0.34±0.011
Total lipids (g L^{-1})	0.0	4.67±0.091	4.47±0.082	4.70±0.122
	0.5	4.63±0.084	4.43±0.091	4.80±0.062
	0.7	4.70±0.101	4.17±0.033 *	4.77±0.194
	0.9	4.73±0.122	4.20±0.101	4.70±0.211

* P<0.05; ** P<0.01; *** P<0.001.

Among the biochemical indicators, the concentration of total lipids is one of the objectives, and it characterizes the metabolism level and the functional state of the body. At the age of 15 months, a decrease in the amount of total lipids in the blood was established in the calves of the experimental groups in comparison with the control group. In the calves of the second and third experimental groups, which were fed Germivit at a dose of 0.7 and 0.9 g kg^{-1}, respectively, the decrease was more noticeable, with differences of 6.04 and 6.71%, respectively. At the same time, the decrease was minimal in the blood of the calves of the first

experimental group, at 0.89%. However, by the age of 18 months, there were no significant differences in the number of lipids between the animals of the control and experimental groups. The indicator was in the range of 4.70 to 4.80 g L^{-1}.

The content of cholesterol in the blood is closely related to lipid metabolism. Feeding of the calves by Germivit had a noticeable effect on blood cholesterol only at the age of 18 months. In control group, the blood cholesterol was 5.29% (P<0.05) higher than the calves of the first experimental group and higher than that of the second and third experimental groups by 4.89% (P<0.05) and 4.51% (P<0.05), respectively.

Bilirubin is an important indicator of the functional state of the liver. No significant differences were recorded in the amount of blood bilirubin among the calves of the experimental groups throughout the experiment. At the same time, it is important to note that while at the age of 15 months, the difference between the animals fed Germivit and the control animals was in the range of 0.50% to 1.01%, then at the age of 18 months it was 4.79% to 5.30%.

The product of the purine metabolism is uric acid, and a change in the amount of uric acid in the blood is a diagnostic sign for kidney disease. Feeding the animals Germivit did not affect the amount of uric acid in their blood throughout the experiment. At the age of 15 months and 18 months, the differences between the representatives of different groups were 0.12% to 0.25% and 0.85% to 1.09%, respectively.

Triglycerides are derivatives of glycerol and higher fatty acids. Elevated triglycerides in the blood may indicate liver disease in animals. Under the influence of Germivit, the animals of the first experimental group showed an 11.43% decrease in blood triglycerides at the age of 15 months, and at the age of 18 months, it reached 10.26% (P<0.05) compared to the control animals. The animals of the second experimental group were lower than the control animals in terms of the quantitative content of triglycerides in the indicated period of study at 14.29% and 10.26% (P<0.05), while the animals of the third experimental group showed a decrease of 14.29% (P<0.05) and 12.82% (P<0.05).

In the experiment with calves, the inclusion of Germivit in the diet contributed with a significant increase in the amount of total protein in the serum. Along with protein, an improvement in carbohydrate metabolism was also observed in the animals. A particularly noticeable increase in blood glucose was observed at 18 months of age in the animals of the experimental groups compared to the control animals. When assessing the lipid metabolism, at the age of 15 months, the amount of total lipids in the blood of the calves of the experimental groups had decreased, when compared with control animals, but at the age of 18 months, it was restored to the control values. During this period, Germivit-fed calves showed a significant decrease in blood cholesterol. Based on the content of bilirubin and triglycerides in the blood, it is possible to assess the clinical condition of the liver in the animals. In experiments, feeding Germivit to calves at doses of 0.5, 0.7 and 0.9 g kg^{-1} live weight reduced the amount of bilirubin and triglycerides in the blood, which may indicate a hepatoprotective effect of the feed additive. Germivit did not adversely affect the kidneys; an indicator of kidney disease is a change in the amount of uric acid and between the control and experimental animals, there were no differences in the amount of uric acid in the blood during all periods of the study.

Al-Yasiri *et al.* (2017) found that the uric acid content, the activity of aspartate aminotransferase and alkaline phosphatase in blood plasma decreased in broiler chickens when phytobiotic was included in their diet. Brogna *et al.* (2014) showed no significant metabolic changes in lambs using phytobiotic. Shawle *et al.* (2016) noted a significant decrease in the concentration of glucose, triglycerides and cholesterol in the serum of broilers with a phytobiotic diet. Karásková *et al.* (2015) found a significant reduction in total cholesterol in broiler chickens. Hao *et al.* (2014) the effect of partially purified red yeast rice extract was determined by Xuezhikang (XZK) for lipoproteins. A decrease in total cholesterol was found.

Autoimmune processes, immune defense in infections, and invasive pathologies are the main reactions in which T-lymphocytes are involved. B-lymphocytes that transform into plasma cells that synthesize antibodies are responsible for humoral immunity in the animal body. At the beginning of the experiment, the content of T-lymphocytes in the

blood of the animals in all test groups was 28.81% to 29.23%, while the content of B-lymphocytes was 13.61% to 14.22%.

By the age of 15 months, the number of T-lymphocytes in the calves' blood in the control group was 29.2±1.07%, which is 3.01% less than in the animals of the first experimental group, 3.22% less than in animals of the second experimental group, and 4.00% (P<0.01) less than in the animals of the third experimental group. By the time the experiment was completed, the difference in the number of T-lymphocytes for the animals in experimental groups was 4.61% (P<0.001), 4.82% (P<0.01), and 4.21% (P<0.001), respectively.

Similar changes were observed when counting B-lymphocytes. In the animals of the first experimental group, the number of B-lymphocytes exceeded the control values at 15 and 18 months of age by 1.21% and 4.02% (P<0.05), respectively, and in the animals of the second experimental group, the differences with animals of the control group were 1.01% and 4.22% (P<0.001), and for the third experimental group, the values were 1.43% (P<0.01) and 4.41% (P<0.01).

Immunoglobulins have an important function in maintaining the body's homeostasis. The largest role in protecting animals against infectious agents belongs to immunoglobulins G (IgG), which includes antibodies that can neutralize viruses and bacteria (Shakhov, 2015). Another factor of the humoral defense of the body against infection is immunoglobulins M (IgM), which is the first formes in the primary immunological reaction and determines the leading position in the formation of immunity in animals (Shakhov, 2015). IgM antibodies are significantly more effective in hemolysis reactions than IgG antibodies.

In the calves of the experimental groups at the age of 9 months, i.e., before starting of the Germivit feeding, the content of immunoglobulins in the blood was at the same level, amounting to IgG 18.65-19.14 g L^{-1} and IgM 3.45-3.57 g L^{-1} (Table 2). However, at the age of 15 months, the calves of the experimental groups showed a significant increase in serum immunoglobulins compared to the control calves. In the first experimental group during this period, the content of IgG increased by 19.81% (P<0.05) and the IgM increased by 12.40% (P<0.05), in animals of the second experimental group these increased by 13.72% (P<0.05)

Table 2. The number of immunoglobulins in the blood of the calves.

Indicators	Germivit (g kg^{-1} live weight)	Age (months)		
		9	15	18
IgG (g L^{-1})	0.0	18.72±0.691	18.94±1.211	19.12±0.742
	0.5	18.65±1.133	22.69±0.842*	23.11±0.922*
	0.7	18.96±0.791	21.54±1.134*	22.87±0.691*
	0.9	19.14±0.942	22.48±0.525*	22.50±1.122*
IgM (g L^{-1})	0.0	3.48±0.194	3.54±0.422	3.49±0.255
	0.5	3.51±0.281	3.98±0.296*	4.16±0.154*
	0.7	3.57±0.322	4.11±0.173*	4.21±0.253*
	0.9	3.45±0.123	3.91±0.157*	4.13±0.183*

* P<0.05

and 16.11% (P<0.05), respectively, and in the third group by 18.73% (P<0.05) and 10.44% (P<0.05), respectively.

By the age of 18 months, the blood of the animals in the experimental groups contained 22.50-23.11 g L^{-1} IgG, which is 17.70-20.81% (P<0.05) more than the control group. In calves of the first experimental group at 18 months of age, the IgM content was higher than in the animals of the control group by 19.22% (P<0.05), the second one 20.65% (P<0.05), and the third one 18.34% (P<0.05).

A study by Kuhn *et al.* (2005) revealed the immunostimulatory effect of Echinacea on sows and their offspring. For

1-day-old piglets, the concentration of IgG and IgA was significantly higher than the controls.

The immune system, along with the nervous and endocrine systems, has an important regulatory function in maintaining the body's homeostasis (Shakhov, 2015).

Under the influence of Germivit in the animals of the experimental groups, the number of T-lymphocytes in the blood significantly increased, indicating an increase in the cellular immunity of the calves. At the same time, the activation of the humoral link of the immune system was also observed due to an increase in the blood of representatives of the experimental groups in the number of B-lymphocytes and, especially, IgG and IgM classes.

Second Experiment

Before the use of the Germivit, the content of red blood cells in the blood of cows of the experimental groups was at the same level with a range of 5.82-5.97×10^{12} L^{-1} (Table 3). Furthermore, 30 and 10 days before the expected calving, no significant differences were found in the number of red blood cells in the cows of the experimental and control groups. The difference was in the range of 0.71-2.11%. However, in the postpartum period, there was a significant increase in the number of red blood cells in the animals of the experimental groups relative to the values for the animals from the control group. Thus, in the cows of the first experimental group, the indicators exceeded the control values by 10.22% (P<0.01) and in the cows of the second experimental group, those exceeded by 10.73% (P<0.01). In the 1-day-old calves obtained from those cows that were additionally fed with Germivit, the number of red blood cells exceeded that from calves born to the cows from the control group by 3.22-4.34%. When studying the morphological composition of blood in the calves at 30 days of age, the difference in the number of red blood cells increased slightly by 9.61% (P<0.05) for the calves of the first experimental group and by 9.01% (P<0.05) for the calves of the second experimental group. The inclusion of Germivit in the diet of pregnant cows did not have a significant effect on the number of leukocytes in the blood of the animals, neither in cows nor in calves. In all the studied periods, the difference between the number of leukocytes in the cows and calves of the experimental groups and the control group was insignificant and unreliable and was in the range of 0.11-2.33% in cows and 0.34-0.62% in the calves obtained from them.

Table 3. Morphological indicators in the blood of the cows and calves.

Indicators	Germivit (g kg^{-1} live weight)	Cows 60 days before calving	Cows 30 days before calving	Cows 10 days before calving	Cows 7 days after calving	One-day calves	30-days calves
Erythrocytes (10^{12} L^{-1})	0	5.86±0.133	5.98±0.164	6.15±0.144	5.89±0.121	6.15±0.131	5.94±0.162
	0.25	5.97±0.091	5.87±0.122	6.23±0.07	6.49±0.140**	6.42±0.09	6.51±0.123*
	0.5	5.82±0.16	6.11±0.081	6.19±0.111	6.52±0.112**	6.35±0.071	6.48±0.075*
White blood cells (10^{9} L^{-1})	0	6.82±0.09	6.94±0.06	5.97±0.081	6.13±0.074	6.72±0.03	6.18±0.054
	0.25	6.93±0.151	6.92±0.122	6.11±0.092	6.10±0.05	6.68±0.071	6.20±0.09
	0.5	6.80±0.071	6.89±0.054	5.89±0.133	6.14±0.083	6.75±0.111	6.15±0.122
Platelets (10^{9} L^{-1})	0	353.26±4.121	348.62±8.124	360.21±7.924	342.82±8.181	324.61±4.151	329.16±7.111
	0.25	329.11±6.15	350.41±7.823	358.41±4.891	346.18±8.14	329.82±7.122	331.15±8.25
	0.5	342.82±5.784	354.11±9.121	361.29±8.15	349.18±7.67	330.16±8.287	330.18±7.651
Hemoglobin (g L^{-1})	0	98.16±2.842	99.82±3.144	100.25±1.221	102.31±4.621	97.62±4.421	99.82±2.69
	0.25	100.11±3.112	98.87±4.141	108.42±4.821*	109.83±3.620*	108.11±3.121**	111.25±2.321**
	0.5	97.87±4.151	100.12±3.97	106.13±2.123*	111.41±2.644*	106.65±4.150**	110.82±4.251**

* P<0.05; ** P<0.01

A similar pattern was established when calculating the number of platelets in the blood of the cows and calves. Therefore, the difference in the number of platelets between the cows of the experimental and control groups 30 days before delivery was 0.51-1.51%, at 10 days, 0.22-0.54%, after calving 0.91-1, 81%, for 1-day-old calves 1.62-1.70%, and for 1-month-old calves 0.34-0.62% (Table 3).

A completely different picture was observed when determining the amount of hemoglobin. The cows of the both experimental groups at 30 days before calving had minimal differences in the amount of hemoglobin in comparison to the cows of the control group (0.31-1.01%). However, 10 days before giving birth, the amount of hemoglobin in the blood of the cows of the first experimental group was 108.42±4.821 g L^{-1}, which is 8.11% (P<0.05) more than in the control animals. Meanwhile, 7 days after calving, the difference was 7.44% (P<0.05). In the cows of the second experimental group, the studied indicator exceeded the control values at 10 days before birth and 7 days after calving by 5.92% (P<0.05) and 8.92% (P<0.05), respectively. The 1-day-old calves of the experimental groups, along with their mothers, exceeded their control peers by 9.12-10.70% (P<0.01) in terms of hemoglobin by 11.01-11.43% (P<0.01) at the age of one month (Table 3).

In other studies, the use of phytobiotics in birds significantly reduces the level of low-density cholesterol lipoproteins in serum and increases the level of hemoglobin and the number of red blood cells (Ademola *et al.,* 2009). Shawle *et al.* (2016) noted that the use of phytobiotics in diets does not change blood indicators, all hematological indicators were included in the norm. However, in this case, phytobiotics increased hemoglobin and the number of red blood cells. There was a significant decrease in serum glucose, triglycerides and cholesterol in the dietary group that consumed phytobiotics.

The lysozyme activity of the blood serum was 17.69-18.11 μg mL^{-1} and the bactericidal activity of blood serum was 47.59-48.14% 60 days before calving in the animals of the experimental groups (Table 4).

Table 4. Humoral factors of natural resistance of the cows and calves.

Indicators	Germivit (g kg^{-1} live weight)	Cows 60 days before calving	Cows 30 days before calving	Cows 10 days before calving	Cows 7 days after calving	One-day after calving	30-days after calving
Lysozyme activity of blood serum (μg mL^{-1})	0	17.72±0.341	18.14±0.155	17.86±0.121	18.12±0.262	9.82±0.122	13.28±0.171
	0.25	18.11±0.41	18.26±0.212	18.76±0.133*	19.15±0.171*	10.41±0.251*	15.41±0.211**
	0.5	17.69±0.49	18.11±0.17	18.82±0.242*	19.26±0.244*	10.24±0.160*	14.49±0.201**
Bactericidal activity of blood serum (%)	0	47.62±0.522	48.12±0.643	47.98±0.321	47.16±0.244	48.64±0.122	49.98±0.162
	0.25	48.14±0.621	50.49±0.233*	50.64±0.444*	49.89±0.170*	50.13±0.29	52.11±0.133*
	0.5	47.59±0.422	51.72±0.131*	49.18±0.182	48.64±0.133*	51.11±0.191*	51.86±0.111

* P<0.05; ** P<0.01.

In the cows of the first and second experimental groups, 30 days before the birth, no differences were found in the lysozyme activity of the blood serum compared with animals from the control group. In the same period, the bactericidal activity of the blood serum significantly increased in the cows of the experimental groups by 2.37-3.60% (P<0.05). The cows of the first experimental group 10 days before delivery exceeded the control animals in terms of serum lysozyme activity by 5.01% (P<0.05) and regarding bactericidal activity by 2.66% (P<0.05), while the animals of the second experimental group were exceeded by 5.32% (P<0.05) and 1.25%, respectively.

After the birth of the cows of the experimental groups, the state of humoral factors of natural resistance continued to remain at a fairly high level. The lysozyme activity of the blood serum was higher than in the control group by 5.74-6.32% (P<0.05) and bactericidal activity was 1.48-2.73% (P<0.05) higher. The 1-day-old calves of the experimental groups exceeded the

lysozyme and bactericidal activity of the blood serum of their control peers by 4.21-6.04% (P<0.05) and 1.49-2.47% (P<0.05), respectively, and at 30 days of age by 9.10-16.01% (P<0.01) and 1.88-2.13% (P<0.05), respectively (Table 4).

When evaluating the cellular factors of the natural resistance (Table 5), of the body of cows under the action of Germivit, 30 days before calving, the phagocytic activity and phagocytic index of blood neutrophils in the animals of all experimental groups did not differ significantly. But 10 days before calving and 7 days after calving, the representatives of the first experimental group recorded an increase in the phagocytic activity of blood neutrophils by 3.25% (P<0.05) and 2.85% (P<0.05), respectively, while the phagocytic index in these study periods increased relative to the control levels by 3.40% (P<0.05) and 5.00% (P<0.05), respectively. Representatives of the second experimental group exceeded the cows of the control group in terms of the phagocytic activity of blood neutrophils by 1.06% (P<0.05) 10 days before calving and by 3.74% (P<0.01) 7 days after giving birth, and in terms of the phagocytic index of blood neutrophils by 2.30% and 3.00% (P<0.05), respectively. In the one-day calves obtained from cows of the experimental groups, the phagocytic index was at the level of the control values and differed insignificantly. On the contrary, the phagocytic activity of blood neutrophils, was 2.73-4.72% (P<0.01) higher than that of their peers from the control. At the age of one month, the calves from the first experimental group had a greater value for the phagocytic activity of neutrophils by 3.63% (P<0.01), and for the representatives of the second experimental group it was by 3.01% (P<0.001). According to the phagocytic index, the monthly calves of the experimental groups were 12.52-13.04% (P<0.01) superior to the monthly calves of the control group (Table 5).

Table 5. Cellular factors of natural resistance of the cows and calves fed daily with Germivit.

Indicators	Germivit (g kg^{-1} live weight)	Cows 60 days before calving	Cows 30 days before calving	Cows 10 days before calving	Cows 7 days after calving	One-day after calving	30-days after calving
Phagocytic activity of neutrophils (%)	0	50.16±1.211	51.82±1.422	49.89±0.711	51.41±1.190	31.46±1.151	41.98±1.271
	0.25	50.45±0.891	52.16±0.741	53.14±0.590*	54.26±0.621*	34.19±0.541**	45.61±0.870**
	0.5	50.79±1.144	52.41±0.890	53.28±0.862*	55.15±0.870**	36.18±0.921**	44.98±0.822**
Phagocytic neutrophil index	0	4.31±0.170	4.39±0.181	4.34±0.190	4.62±0.711	1.29±0.121	3.68±0.090
	0.25	4.37±0.211	4.43±0.160	4.49±0.244*	4.85±0.343*	1.32±0.180	4.16±0.144**
	0.5	4.29±0.422	4.50±0.212	4.44±0.160	4.76±0.222*	1.30±0.150	4.14±0.121**

* - P<0.05; ** - P<0.01.

The bacteriostatic and bactericidal action of body fluids is provided by the humoral factors of natural resistance. During the experiments, the lysozyme and bactericidal activity of the blood serum was highest in the cows of the experimental groups both before and after calving. Similar changes were recorded in the assessment of the cellular factors of natural resistance. The phagocytic activity of blood neutrophils was also higher in cows from the experimental groups.

The use of different plants in phytobiotics had a significant effect on total cholesterol, total antibody titers and immunoglobulin G (IgG). However, there was no significant effect of the supplements on the production of IgM. Differences in results may be due to numerous factors, for example, the type and part of the plant used, harvest time, methods for preparing phytogenic additives and methods for extracting herbs (Yang *et al.,* 2009).

When evaluating the mineral metabolism in cows, those Germivit-fed did not lead to significant changes in blood levels of calcium, phosphorus, and magnesium 30 days before birth. The blood of the cows of the experimental groups 10 days before calving had 4.81% and 2.82% more magnesium, 2.93% and 1.11% more calcium, and 5.14% (P<0.05) and 7.61% (P<0.05) more phosphorous than cows without supplement. In the postpartum period, a significant increase in the studied mineral substances in the blood of cows of the experimental groups was found. In terms of the magnesium content, the cows of the first

experimental group significantly exceeded the values for the control animals by 7.72% (P<0.05), calcium by 9.63% (P<0.05), and phosphorus by 8.01% (P<0.05). The animals of the second experimental group had 5.84% (P<0.05) more magnesium, 10.81% more calcium, and 13.22% (P<0.05) more phosphorus in the blood. The calves obtained from the cows of the experimental groups at 1-day-old and 1-month-old did not differ from the calves of the control group of the same ages in terms of the blood content of magnesium, calcium, and phosphorus (Table 6).

Table 6. The mineral composition of the blood of the cows and calves.

Indicators	Groups	Cows 60 days before calving	Cows 30 days before calving	Cows 10 days before calving	Cows 7 days after calving	One-day after calving	30-days after calving
Magnesium (mmol L^{-1})	Control	1.12±0.032	1.10±0.026	1.05±0.013	1.04±0.017	1.06±0.02	1.08±0.019
	First experimental	1.07±0.014	1.09±0.018	1.10±0.044	1.12±0.023*	1.09±0.012	1.12±0.022
	Second experimental	1.08±0.024	1.11±0.02	1.08±0.017	1.10±0.018*	1.05±0.017	1.10±0.015
Calcium (mmol L^{-1})	Control	2.64±0.111	2.69±0.082	2.67±0.137	2.59±0.096	2.42±0.063	2.47±0.088
	First experimental	2.68±0.144	2.71±0.091	2.75±0.171	2.84±0.083*	2.40±0.011	2.45±0.092
	Second experimental	2.63±0.121	2.60±0.08	2.70±0.092	2.87±0.063*	2.38±0.121	2.50±0.101
Phosphorus (mmol L^{-1})	Control	1.58±0.033	1.60±0.044	1.56±0.032	1.59±0.082	1.49±0.033	1.51±0.07
	First experimental	1.60±0.041	1.59±0.07	1.64±0.071*	1.72±0.044*	1.51±0.091	1.48±0.09
	Second experimental	1.57±0.044	1.62±0.052	1.68±0.085*	1.80±0.035*	1.47±0.052	1.50±0.063

* P<0.05.

The composition of Germivit includes various vitamins, and it is known that many vitamins have a positive effect on immunity. Under the influence of Germivit, an increase in the mineral content in the cows' blood was observed; in particular, the maximum difference was seen in the content of magnesium, calcium, and phosphorus in the blood after birth, which was associated with the presence of these minerals in the feed supplement. The content of transamination enzymes in the blood of the cows of the experimental groups 60 days before calving was 31.41-33.26 U L^{-1} for ALT (alanine aminotransferase) and 58.54-59.18 U L^{-1} for AST (aspartate aminotransferase). After 30 days from the start of feeding cows with Germivit, the blood levels of these enzymes changed slightly. A significant decrease in the blood of the cows of the experimental groups 10 days before birth was observed in terms of the amount of ALT by 4.81-7.61% (P<0.05) relative to the control values. The decrease in the amount of AST was less significant and amounted to 1.82-2.91%. After calving, the amount of ALT and AST was lower in the cows of the first experimental group than in the

Table 7. The content of transamination enzymes in the blood of the cows and calves.

Indicators	Groups	Cows 60 days before calving	Cows 30 days before calving	Cows 10 days before calving	Cows 7 days after calving	One-day after calving	30-days after calving
Alanine aminotransferase (U L^{-1})	Control	31.41±0.266	31.62±0.191	30.58±0.263	31.48±0.232	28.98±0.241	29.97±0.174
	First experimental	33.26±0.181	31.70±0.322	29.11±0.151*	30.32±0.122	28.14±0.422	30.19±0.285
	Second experimental	31.98±0.311	31.59±0.414	28.41±0.290*	30.46±0.545	29.12±0.265	30.41±0.255
Aspartate aminotransferase (U L^{-1})	Control	58.97±0.751	59.18±1.161	58.48±1.144	59.11±1.122	57.48±0.823	57.91±0.92
	First experimental	59.18±0.852	57.11±0.89	57.43±0.433	56.92±0.87	58.03±1.141	58.16±1.133
	Second experimental	58.54±0.491	58.41±0.754	56.82±0.621	57.12±1.15	57.39±0.444	58.21±0.82

* P<0.05.

cows of the control group by 3.81%, and for the cows of the second experimental group, it was 3.33% and 3.43% lower, respectively. In the calves of the control and experimental groups, no differences were found in the content of enzymes in the blood (Table 7).

There was a decrease in the blood of animals from the experimental groups in terms of the content of transamination enzymes, especially alanine aminotransferase, which are markers of liver damage. Hence, a decrease in these enzymes indicates a hepatoprotective effect of Germivit. Feeding Germivit to pregnant cows contributed to the birth of more viable offspring, whereby of the 10 calves obtained from the cows of the control group, a gastrointestinal pathology was recorded in four animals. Despite etiotropic and symptomatic treatment, two calves died. In the second experimental group, one calf in mild form became ill. In the group of calves obtained from the cows of the first experimental group, no incidence of calf mortality was observed (Table 8).

Table 8. Clinical indicators of the calves.

Indicators	Groups		
	Control	First experimental	Second experimental
The number of sick calves	4	0	1
The number of dead calves	2	0	0

The state of health of cows has great importance for obtaining healthy and viable young stock. The correlation among the immunological status of young calves, the level of metabolism and natural resistance of mother cows has been scientifically proven by several authors (Kulmakova and Mudarisov, 2019; Kulmakova *et al*., 2019).

Feeding with Germivit to pregnant cows contributed to an improvement in the morphological composition of the blood of their offspring, whereby a significant increase in humoral and cellular resistance factors was observed at the age of 1-day-old and 1-month-old, which in turn contributed to a decrease in the incidence of mortality among the calves.

A positive effect of Germivit on the immuno-biochemical status of animals has been previously established in experiments on calves during the dairy period of growth. In animals, there is an improvement in the clinical condition, which is confirmed by the results of blood monitoring. According to Donnik *et al.* (2010), in the blood of the Germivit-fed cows, the phosphorus-calcium ratio was normalized, the cholesterol content decreased by 13%, the urea content increased, evidencing a normalization of liver function. These results are consistent with this study.

There are currently many herbal preparations that have a positive effect on the functional state and productivity of animals (Patel *et al*., 2016; Shawle *et al*., 2016; Valenzuela-Grijalva *et al*., 2017). The advantage of Germivit compared to its analogs is the low cost and wide availability of its raw materials, the absence of side effects, the safety for animals in cases of overdose, and the pronounced positive effect on the immuno-biochemical status of the animal organism shortly after starting its use. Therefore, Germivit is a good alternative to antibiotics in the context of animal husbandry.

CONCLUSION

The research results confirm the beneficial effects of Germivit in cattle. Therefore, in calves fed with Germivit, an improvement in the metabolism, an increase in immune status, and a normalization of liver function were observed. In cows, an improvement in the morphological composition of blood and the mineral metabolism was observed, while the humoral and cellular factors of natural resistance also increased. Calves born from cows that were fed with Germivit during pregnancy showed a high state of immunity. There was no mortality among the calves of the experimental groups. It should be noted that the morphological, biochemical, and immunological parameters of the cows and calves of the experimental groups did not depend on the dose of Germivit received.

To sum up, the use of Germivit in cattle of different ages normalizes the metabolism, increases the immune

status of the animal, and improves the functional state of the liver. The research showed uniformly positive effect of Germivit on experimental animals. On the basis of existing results and economic viability, more appropriate dose was 0.5 g kg^{-1} in the first experiment (in growing calves) and 0,25 g kg^{-1} in the second one (in pregnants cows).

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Revista Facultad Nacional de Agronomía

Larvicidal activity of vegetable oils against *Aedes aegypti* larvae

UNIVERSIDAD NACIONAL DE COLOMBIA

Actividad larvicida de aceites vegetales contra larvas de *Aedes aegypti*

https://doi.org/10.15446/rfnam.v74n2.91486

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Keywords:
Alternative control
Dengue
Mosquito
Vegetable larvicide

ABSTRACT

Aedes aegypti L. is the mosquito vector of yellow fever, dengue, zika, and chikungunya viruses. The prevention and control of such diseases usually rely on the use of chemicals, that can cause harm to human health and the environment. Vegetable oils with larvicidal activity are used as an alternative tool to control this insect. This study aimed to evaluate the larvicidal activity of vegetable oils from *Caryocar coriaceum*, *Mauritia flexuosa*, *Carapa guianensis, Copaifera langsdorffii, Ricinus communis* and *Cocos nucifera* against *A. aegypti* larvae. The experiment was divided into two bioassays. In the first, a completely randomized design was used with seven treatments (six vegetable oils at 500 ppm and one control with four replications). The number of dead larvae was evaluated 24, 48, 72, 96, and 120 h after exposure. In the second bioassay, the most efficient vegetable oils from the first bioassay (*C. coriaceum* and *M. flexuosa*) were used at the concentrations of 0, 500, 1000, 1500, 2000, and 2500 ppm, with four replications. The number of dead larvae was evaluated according to the first bioassay. All oils used had larvicidal activity on third-instar stage larvae of *A. aegypti*, with greater efficiency 120 h after exposure. The oils of *C. coriaceum* and *M. flexuosa* at 2500 ppm had the best efficacy in the larvae control. The LD_{10}, LD_{50}, and LD_{90} of *M. flexuosa* oil recommended for controlling larvae are 234, 648, and 1794 ppm, respectively.

Palabras clave:
Control alternativo
Dengue
Mosquito
Larvicida vegetal

RESUMEN

Aedes aegypti L. es el mosquito que transmite el virus de la fiebre amarilla, el dengue, el zika y el chikungunya. La prevención y el control de tales enfermedades generalmente dependen del uso de productos químicos, que causan daños al hombre y al medio ambiente. Por ello, los aceites vegetales con acción larvicida se utilizan como alternativa para controlar este insecto. El objetivo de este trabajo fue evaluar el potencial larvicida de aceites vegetales de *Caryocar coriaceum*, *Mauritia flexuosa*, *Carapa guianensis*, *Copaifera langsdorffii*, *Ricinus communis* y *Cocos nucifera* contra *A. aegypti*. El experimento se dividió en dos bioensayos. En el primero se realizó un diseño completamente al azar con siete tratamientos (seis aceites vegetales a 500 ppm y un control y cuatro repeticiones). Se evaluó el número de larvas muertas a las 24, 48, 72, 96 y 120 h de exposición. En el segundo bioensayo, se utilizaron los aceites vegetales más eficientes (*C. coriaceum* y *M. flexuosa*) en concentraciones 0, 500, 1000, 1500, 2000 y 2500 ppm, con cuatro repeticiones. El número de larvas muertas se evaluó según el primer bioensayo. Todos los aceites utilizados tienen efecto larvicida sobre larvas de *A. aegypti* de tercer estadio, con mayor eficacia a las 120 h de exposición. Los aceites de *C. coriaceum* y *M. flexuosa* mostraron mejor eficacia en el control de larvas, siendo la dosis de 2500 ppm la más recomendada. El LD_{10}, LD_{50} y LD_{90} del aceite de *M. flexuosa* recomendado para controlar larvas son 234, 648 y 1794 ppm, respectivamente.

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Received: February 19, 2021; Accepted: March 26, 2021
Rev. Fac. Nac. Agron. Medellín 74(2): 9563-9570. 2021
ISSN 0304-2847 / e-ISSN 2248-7026

Arboviruses are viruses transmitted by arthropods that have received high public health attention worldwide (Weaver and Reisen, 2010). The most important diseases caused by arboviruses that infect humans are dengue, zika, yellow fever, and chikungunya (Shepard *et al*., 2014). These diseases are vector-transmitted by the insect *Aedes aegypti.* However, *Aedes albopictus* is also a vector of the dengue virus (Kraemer *et al*., 2015). These diseases caused by viruses are one of the main global health concern that has increased dramatically due to their rapid geographical spread, high disease burden, and distribution (Leta *et al*., 2018). The spread of the insect vector has reached areas beyond the tropics in recent years, causing a significant problem not only in undeveloped countries (Kraemer *et al*., 2015; Chadee and Martinez, 2016).

Artificial reservoirs such as old discarded tires, plant pots, uncovered water tanks, and untreated pools are the main oviposition sites for *A. aegypti*, where the female mosquitoes are attracted to deposit their eggs (Muktar *et al*., 2016). The *Aedes* larvae can be distinguished from other genera with the naked eye due to their short siphon, used to breathe oxygen that is kept above the water surface while the rest of the body remains vertically immersed (Muktar *et al*., 2016). Males develop faster than females, and if temperature is cold, *A. aegypti* can stay in the larval stage for months, as long as the water supply is maintained (Muktar *et al*., 2016).

Several countries have developed plans to contain *A. aegypti*, with the aim of reducing the infestation rates and the impacts caused by the viruses transmitted (Nash *et al*., 2017). Investing in measures that intend to control the transmitting vector and reduce their proliferation is as fundamental as the development of vaccines and diagnostic methods (Zara *et al*., 2016).

According to the Ministry of Health of Brazil (MHB, 2019), in the first 11 weeks of 2018, 62.900 cases of dengue were registered in this country, and in 2019 this number increased by 264.1%, with a total of 229.064 cases in the same period of the year. Different insecticides (organophosphates and pyrethroids) and larvicides (organophosphates and growth regulators) are used to control *A. aegypti* in Brazil due to the increasing resistance of this vector (Augusto *et al*., 2016). However, the use of these products can cause harmful effects on human health and the environment (Mendes *et al*., 2017).

The increasing resistance of mosquito populations has led to studies on alternative tools for vector control. From this perspective, vegetable compounds are a promising and environmentally safe strategy to prevent larvae survival due to the bioactive properties of their compounds (Marangoni *et al*., 2012; Garcez *et al*., 2013).

The effectiveness of the bioactive extracts, oils, and isolated compounds obtained from vegetables are being researched with great efforts, increasing the list of plants with medicinal and pest control properties (Garcez *et al*., 2013). These plant species are a promising source for vector control due to their low toxicity to humans and other living organisms, low concentrations needed, and no cumulative effect on the environment (Garcez *et al*., 2013).

Natural compounds with larvicidal or insecticidal effects are an alternative for A. *aegypti* control since these products are generally less harmful to non-target organisms, biodegradable, efficient, and with low cost (Mendes *et al*., 2017). The interest in its medicinal uses has attracted the attention from traditional communities (Barros *et al*., 2014). However, there is a lack of information in the literature about its use as a larvicide. In this context, this study aimed to evaluate the larvicidal activity of vegetable oils from *Caryocar coriaceum, Mauritia flexuosa, Carapa guianensis, Copaifera langsdorffii, Ricinus communis* and *Cocos nucifera* against *A. aegypti* larvae.

MATERIALS AND METHODS

Installation of traps

To obtain *A. aegypti* eggs, oviposition traps (ovitraps), black plastic pots (400 mL capacity) with 10% hay extract, and pressed wood pallet (type Eucatex) were installed in residences of Crato and Juazeiro do Norte, Ceará, Brazil. The traps were collected 5 days after installation, and straws with the eggs were immersed in tap water for larvae hatching. The hatched larvae

remained in the trays with water and were fed with fish food (Alcon Pet, Santa Catarina, Brazil) until the third-instar larval (L3) (Silva *et al*., 2017).

Vegetable oils obtaining and application

The vegetable oils were purchased at local family farmers' fairs in the municipalities of Crato and Juazeiro do Norte-CE. The oils were selected according to their medicinal use and their sale frequency at the fairs. *C. coriaceum* oil is composed of oleic acid (50.2%) and palmitic acid (44.3%), as main components (Croda do Brasil, 2002). *M. flexuosa* oil has 79.6% oleic acid, 16.1% palmitic acid and 1.3% linoleic acid (Soares *et al*., 2020). *C. guianensis* oil has myristic acid (0.04%), linolenic acid (0.2%), behenic acid (0.3%), palmitoleic acid (0.8%), arachidic acid (1.4%), stearic acid (8.9%), linoleic acid (9.5%), palmitic acid (27.7%) and oleic acid (50.9%) (Azevedo *et al*., 2017). *C. langsdorffii* oil is composed of 55 to 60% of sesquiterpene acids (Cascon, 2000). *R. communis* oil is composed of 84 to 91% ricinoleic acid, oleic acid (3.1-5.9%), linoleic (2.9-6.5%), steric (1.4-2.1%) and palmitic (0.9-1.5%) (Embrapa, 2017) and *C. nucifera* oil is composed of lauric acid (45-53%), myristic (16-21%), palmitic (7-10%), caprylic (5-10%) and capric (5-8%) and the main triacylglycerol is trilaurine (22.2–23.9%), which consists of a glycerol esterified with three lauric acids (Silva *et al*., 2020).

The study was carried out at the Agricultural Entomology Laboratory, Center for Agricultural and Biodiversity Sciences, Federal University of Cariri, from May to June of 2019.

A two-step assay was carried out. In the first step, the most toxicologically efficient vegetable oils against *A. aegypti* larvae were selected. In the second step, the lethal doses of the selected oils were determined. The assays were set under environmental conditions, with temperature and relative humidity monitored with a Thermo hygrometer.

First bioassay

A completely randomized experimental design was used with seven treatments: pequi (*Caryocar coriaceum* Wittm.), buriti (*Mauritia flexuosa* L.), andiroba (*Carapa guianensis* Aubl.), copaiba (*Copaifera langsdorffii* Desf.), castor (*Ricinus communis* L.), and coconut (*Cocos nucifera* L.) vegetable oil, and water used as the control treatment, with four replications with 10 larvae at the L3 instar for each replication.

The doses of the treatments used were adjusted at 500 ppm plus Tween® 20 used as a surfactant to aid in the dilution of the oils in water. In the control treatment, distilled water and Tween® 20 were used. The larvae were submitted to the treatments for 24, 48, 72, 96, and 120 h of exposure. Mortality was assessed when dead larvae did not react to the mechanical stimulus of fine-pointed forceps.

Second bioassay

After selection of the oils with the best larvicidal effect against *A. aegypti* (oils from *C. coriaceum* and *M. flexuosa*), a second bioassay was set up. A completely randomized experimental design was used in a 2x6 factorial scheme. The treatments consisted of six concentrations for each oil (*C. coriaceum* and *M. flexuosa* - 0, 500, 1000, 1500, 2000, and 2500 ppm). The control treatment and assessment of larvae mortality followed the same procedures as described in the first bioassay.

Mortality efficiency

The larvae mortality efficiency was determined using the following equation (Abbott, 1925): E (%)=[((Nc-Nt)/Nc))x100], where, E=efficiency; Nc=number of alive individuals in the control treatment; Nt=number of alive individuals in the treatments.

Statistical analysis

Data were submitted to analysis of variance (ANOVA) using the R software. Means of quantitative factors were compared by the Tukey test, and means of qualitative factors by regression analysis at 5% probability. Probit analysis (Finney, 1971) was performed in the second assay to obtain the LD_{10}, LD_{50}, and LD_{90} through the PoloPlus 1.0 software, with 5% confidence interval level significance.

RESULTS AND DISCUSSION

After contact of *A. aegypti* larvae with the vegetable oils, their movements became slow, in addition to tremors and convulsions. In tests with organophosphates and plant products against larvae of *A. aegypti* and *A. Albopictus*, the same symptoms were observed (Kanis *et al*., 2012). The

oil of *M. flexuosa* caused the highest mortality of the larvae after 24 h and was only statistically different from *C. langsdorffii*. The oils *of C. coriaceum, C. guinanensis, R. communis,* and *C. nucifera* were statistically equal. The oil of *C. langsdorffii* caused the lowest mortality and was statistically different from the other oils. During the other four exposure periods, no statistical difference between the treatments was observed (Table 1). The ethanolic extract of *Azadichta indica* at the dose of 50 mg L^{-1} caused 93% mortality of *A. aegypti* larvae after 72 h of exposure (Manzano *et al*., 2020).

Table 1. Mortality (number of dead larvae) and efficiency (%) of *Aedes aegypti* larvae submitted to the medicinal oils within each exposure period at the dose of 500 ppm.

Medicinal oils	24 h	48 h	72 h	96 h	120 h
Caryocar coriaceum	2.25 ab (20.51)	5.50 a (78.95)	8.25 a (89.74)	9.00 a (89.74)	9.75 a (100)
Mauritia flexuosa	3.75 a (35.89)	6.75 a (52.63)	7.75 a (76.31)	8.50 a (81.58)	8.25 a (84.21)
Carapa guianensis	2.25 ab (20.51)	6.00 a (57.89)	6.75 a (57.89)	6.75 a (65.78)	6.75 a (65.78)
Copaifera langsdorffii	0.50 b (2.56)	5.50 a (52.63)	6.50 a (63.16)	7.25 a (71.05)	8.25 a (81.58)
Ricinus communis	2.00 ab (17.95)	4.25 a (39.47)	6.50 a (63.16)	6.50 a (63.16)	7.00 a (68.42)
Cocos nucifera	3.25 ab (30.77)	6.25 a (60.53)	7.75 a (76.31)	8.25 a (81.58)	8.50 a (81.58)
Control	0.25 b (0)	0.50 b (0)	0.50 b (0)	0.50 b (0)	0.50 b (0)

Means followed by the same letter in the columns are not statistically different by the Tukey test at 5% probability.

According to Abbot's efficiency test, represented by the values in parentheses, the oil of *M. flexuosa* was the most efficient after 24 h of exposure, with almost 36%, followed by *C. nucifera* and *C. coriaceum* with 30.77 and 20.51%, respectively (Table 1). The oil of *C. nucifera* caused a 50% mortality in *A. aegypti* larvae under the same conditions and for the same exposure period, at 500 ppm (Fazal *et al*., 2013).

Only *R. communis* oil did not surpass 50% mortality after 48 h of exposure. After 96 h the oil of this plant, in a dose of 20% of the product, caused a larvae mortality of 45% (Neves *et al*., 2014). This oil is mostly composed of 90% ricinoleic acid, and although it causes low mortality in *A. aegypti* larvae, it is effective in controlling cashew white fly nymphs (*Aleurodicus cocois* Curtis), with an efficiency of over 90% between 48 and 120 h after application, using 2% oil (Silva *et al*., 2007).

The *C. coriaceum* oil provided the best mortality efficiency 48 h after exposure, with a 60% increase compared to the previous period. The *C. guianensis* oil provided the lowest progression in larvae mortality, with the lowest efficiency value compared to the other oils. However, the repellent activity of this oil against adults of *A. aegypti* was reported (Bueno and Andrade, 2010).

The *C. langsdorffii* oil caused a 90% mortality 96 h after exposure at 200 ppm (Trindade *et al*., 2013). In the present study, mortality of more than 80% occurred 120 h after exposure at 500 ppm. These differences are probably due to the oil purity since some extractors usually dilute the product before sale. The higher nutritional quality and commercial value of authentic vegetable oils have led to their adulteration with the use of low-grade (seed oils), refined pomace, or esterified oils (Popescu *et al*., 2015). Adulteration in other high-price oils is still the biggest source of agricultural fraud problems (Zhang *et al*., 2014).

The highest efficiency was obtained by the *C. coriaceum* oil, followed by *M. flexuosa* and *C. nucifera* oils, which provided

the same efficiency 72 and 96 h after larvae exposure. After 120 h, only *C. coriaceum* caused 100% mortality, followed by *M. flexuosa* oil with 84.21% efficiency. The last two oils were selected for the second assay due to their higher larvae mortality in the dose of 500 ppm, 120 h after exposure. The *C. coriaceum* is a plant of the Caryocaraceae family native to the Brazilian Cerrado. Its fruits are very rich in oil, proteins, and carotenoids. The oil, which is composed mainly of unsaturated fatty acids, is considered of great quality. Fatty acids, carotenoids, and ascorbic acid are found in the chemical composition of *M. flexuosa*.

According to the analysis of variance, in the second assay (Table 2), an interaction between *C. coriaceum* and *M. flexuosa* oils was observed within the doses and the exposure periods. No significant difference between the mortality rates was observed. However significant interaction was obtained between oil and period, and concentration and period.

Table 2. Analysis of variance of the effect between oils, doses and exposure periods.

Variation Factor	Degrees of freedom	Mean Square
Oil	1	8.07**
Dosages	5	318.35**
Exposure period	4	334.31**
Oil x Dosage	5	1.63^{ns}
Oil x Period	4	7.19**
Concentration x Period	20	14.13**
Oil x Dosage x Period	20	0.75^{ns}
Residue	180	1.05

* Significant at 5% probability; ** significant at 1% probability; ^{ns} no significant.

As observed in the interaction with the oils and exposure time, a significant difference was observed at 5% probability. The *C. coriaceum oil* provided the highest means (Table 3). Similar behavior was observed 48 h after exposure of the larvae to the treatments. For the remaining exposure periods, no statistical difference was observed between the two treatments. Both *C. coriaceum* and *M. flexuosa* oil effectively controlled *A. aegypti* larvae when exposed for 72, 96, and 120 h.

Table 3. Larvicidal effect (number of dead larvae) of *Caryocar coriaceum* and *Mauritia flexuosa* oils within each exposure periods.

Medicinal Oils	Exposure periods (h)				
	24	48	72	96	120
Caryocar coriaceum	1.87 a	5.50 a	6.54 a	7.16 a	7.70 a
Mauritia flexuosa	0.70 b	4.25 b	6.62 a	7.50 a	7.87 a

Means followed by the same letter in the columns are not statistically different by the Tukey test at 5% probability.

The total mean doses of the *C. coriaceum* and *M. flexuosa* oils when submitted to regression analysis (Figure 1), provided the effectiveness of the larvicidal effect in relation to exposure time, and at the period of 120 h after exposure, both oils caused higher mortality. Thus, it could be presumed that the 2-degree polynomial regression curves represent well the toxic activity of these oils against *A. aegypti*.

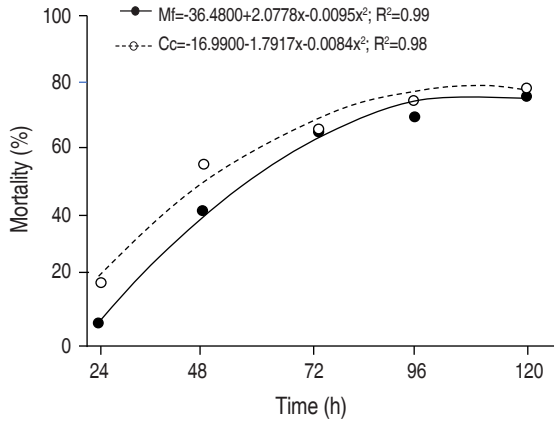

Figure 1. Mortality of *Aedes aegypti* larvae under application of *Caryocar coriaceum* and *Mauritia flexuosa* oils. Mf = *Mauritia flexuosa*; Cc = *Caryocar coriaceum*.

According to the interaction analysis between the doses and the exposure time (Table 4), it was observed that regardless of the oil used, the dose of 2500 ppm promoted the highest mortality with 24 h after exposure. The same occurred 48 h after exposure; nevertheless, the doses of 500, 1000, 1500, and 2000 ppm did not statistically differ at 5% probability.

The doses of 2000 and 2500 ppm promoted the highest mortality rates, with no statistical differences when the larvae were exposed for 72, 96, and 120 h. Therefore, the dose increase was proportional to the increase in the number of dead larvae. The dose of 2500 ppm and the exposure period of 120 h were the most appropriate conditions to cause the highest mortality rate of *A. aegypti* larvae.

Table 4. Larvae mortality (number of dead larvae) of *Aedes aegypti* under doses of *Caryocar coriaceum* and *Mauritia flexuosa* oils and exposure period.

Dosages (ppm)	Mortality (number of dead larvae)				
	Exposure periods (h)				
	24	48	72	96	120
0	0.00 c	0.00 c	0.00 c	0.00 c	8.00 d
500	0.75 b	4.75 b	7.00 b	7.88 b	8.38 c
1000	1.25 b	5.25 b	7.50 ab	8.00 b	8.50 bc
1500	1.38 b	5.88 b	8.50 a	9.50 a	10.00 a
2000	1.50 ab	5.87 b	7.75 a	9.00 a	9.88 a
2500	2.88 a	7.50 a	8.75 a	9.63 a	10.00 a

Means followed by the same letter in the columns are not statistically different by the Tukey test at 5% probability.

The larvicidal activity of the *C. coriaceum* ethanolic extract at 250 ppm was evaluated against *A. albopictus* larvae. A mortality of 65 and 95% was observed 24 and 48 h after exposure, respectively. All larvae were dead 24 h after exposure when the extract was used at 1000 ppm (Viana *et al*., 2018). In the present study higher doses were required to cause mortality to the *A. aegypti* larvae. The larvicidal activity of *Nigella sativa* oil against *A. aegypti* had LC_{90} at 523.5 ppm after 12 h of larvae exposure (Raj *et al*., 2015).

The minimum (LD_{10}), median (LD_{50}), and maximum (LD_{90}) doses of *M. flexuosa* oil were able to cause mortality in *A. aegypti* larvae (Table 5). It was not possible to perform the Probit test for the *C. coriaceum* oil treatment due to the lack of variation in mortality within the concentrations since the dose of 500 ppm was enough to kill 100% of the larvae.

A LD_{50} value of 620 ppm was obtained for *Artemisia abrotamum* leaf extract, a value relatively close to the

value obtained in the present study. However, for *Curcuma longa* L. and *Melaleuca leucadendron* L. oils, the LD_{50} values were lower, 113 and 120 ppm, respectively (Leyva *et al*., 2008). Anees *et al*. (2008) found that the *Ocimum sanctum* L. oil had a lethal concentration of 425.94 ppm against *A. aegypti*, while for larvae *of Culex quinquefasciatus*, the LD_{50} was 592.60 ppm. Different results were obtained for *Croton tiglium*, *Cascabela thevetia*, *Ricinus communis*, and *Datura stramonium* seed oils. The larvicidal activity of this oil against *A. aegypti* had LC_{50} of 82.08, 95.19, 80.83 and 88.69 ppm, respectively, 24 h after larvae exposure (Borah *et al*., 2012).

Table 5. Lethal doses (LD) of *Mauritia flexuosa* oil against *Aedes aegypti* larvae.

Species	LD_{10}	LD_{50}	LD_{90}
		(ppm)	
M. flexuosa	234	648	1794
Confidence intervals (0.05)	-	-	(1049 - 3067)

CONCLUSIONS

The medicinal *Caryocar coriaceum, Mauritia flexuosa, Carapa guianensis, Copaifera langsdorffii, Ricinus communis*, and *Cocos nucifera* oils have a larvicidal activity on third-instar stage larvae of *Aedes aegypti*, with greater efficiency 120 h after exposure.

The *Caryocar coriaceum* and *Mauritia flexuosa oils* provided the highest control efficacy of *Aedes aegypti* larvae, at 2500 ppm. The recommended LD_{10}, LD_{50}, and LD_{90} of *Mauritia flexuosa* oil to control *Aedes aegypti* larvae are 234, 648, and 1794 ppm, respectively.

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Effect of different concentrations of indole butyric acid, putrescine and hydrogen peroxide on stem cuttings of the rootstock GF677(*Prunus amygdalus* × *Prunus persica*) according to the cutting season

Efecto de diferentes concentraciones de ácido indol butírico, putrescina y peróxido de hidrógeno en el enraizamiento de esquejes de tallo de durazno GF677 (*Prunus amygdalus* × *Prunus persica*) según las temporadas de corte

https://doi.org/10.15446/rfnam.v74n2.92414

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ABSTRACT

The rootstock GF677 is an interspecific hybrid with an important economic and horticultural value. In this research, the effect of indole butyric acid (IBA) in combination with putrescine (Put) and hydrogen peroxide (H_2O_2) on rooting of GF677 semi-hardwood stem cuttings in three cutting seasons (July, March and October) was investigated. Treatments as IBA (0, 1000, 2000 and 3000 mg L^{-1}), Put (0, 800, 1600 and 3200 mg L^{-1}) and H_2O_2 (1.5, 3 and 6% w/v) were included. The results showed that in July cuttings, the highest levels of callogenesis were observed in IBA treated cuttings in both concentrations of 1000 and 2000 mg L^{-1}. The rooting was very low in July cuttings, while the highest percentage of rooting (14%) was observed in the combination of 2000 mg L^{-1} IBA+ 3% H_2O_2. In March, the cuttings treated by 1000 mg L^{-1} IBA+800 mg L^{-1} Put and 1000 mg L^{-1} IBA+1600 mg L^{-1} Put revealed the highest percentages of callus formation 83.31 and 83.33%, respectively. In these cuttings, the highest percentage of rooting (63.88%) was gained at 2000 mg L^{-1} IBA+3200 mg L^{-1} Put. The application of 1000 mg L^{-1} IBA+800 mg L^{-1} Put increased root fresh weight. In cuttings prepared in October, only 800 mg L^{-1} Put caused callus formation in more than 55% of the cuttings. The rooting of cuttings at this time was as low as the July cuttings, whereas the highest rooting percentage was observed in cuttings treated with IBA at a concentration of 1000 mg L^{-1}. Overall, the experiment showed that the season of the cutting and the treatments with IBA+Put or H_2O_2 could improve rooting properties of the rootstock GF677.

Keywords:
Adventitious rooting
Carbohydrate
Co-factors
Semi-hardwood cuttings
Woody cuttings

RESUMEN

El portainjerto GF677 es un híbrido interespecífico con un importante valor económico y hortícola. En este estudio, se investigó el efecto del ácido indol butírico (IBA) en combinación con putrescina (Put) y peróxido de hidrógeno (H_2O_2) en el enraizamiento de esquejes de tallos de madera semidura GF677 en tres temporadas de corte (julio, marzo y octubre). Los tratamientos incluyeron IBA (0, 1000, 2000 y 3000 mg L^{-1}), Put (0, 800, 1600 y 3200 mg L^{-1}) y H_2O_2 (1.5, 3 y 6% p/v). Los resultados mostraron que en los esquejes de julio, la callogénesis más alta se observó en los esquejes tratados con IBA en ambas concentraciones de 1000 y 2000 mg L^{-1}. El enraizamiento fue muy bajo en los esquejes de julio y el mayor porcentaje de enraizamiento (14%) se observó en la combinación de IBA 2000 mg L^{-1}+H_2O_2 3%. En los esquejes de marzo, el mayor porcentaje de formación de callos se alcanzó en los tratados con IBA 1000 mg L^{-1}+Put 800 mg L^{-1} e IBA 1000 mg L^{-1}+Put 1600 mg L^{-1}, 83.31 y 83.33%, respectivamente. En estos esquejes, el mayor porcentaje de enraizamiento (63.88%) se obtuvo con IBA 2000 mg L^{-1}+Put 3200 mg L^{-1}. La aplicación de IBA 1000 mg L^{-1}+Put 800 mg L^{-1} aumentó el peso fresco de la raíz. En esquejes preparados en octubre, el Put solo a una concentración de 800 mg L^{-1} provocó la formación de callos en más del 55% de los esquejes. El enraizamiento de los esquejes en este momento era tan bajo como los esquejes de julio y el mayor porcentaje de enraizamiento se observó en los esquejes tratados con IBA a una concentración de 1000 mg L^{-1}. En general, el experimento mostró que la temporada de corte y el tratamiento con IBA+Put o H_2O_2 podría mejorar las propiedades de enraizamiento de los esquejes de GF677.

Palabras clave:
Enraizamiento adventicio
Carbohidratos
Co-factores
Esquejes de madera semidura
Esquejes leñosos

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Received: March 03, 2021; Accepted: April 01, 2021
Rev. Fac. Nac. Agron. Medellín 74(2): 9571-9582. 2021

ISSN 0304-2847 / e-ISSN 2248-7026

Vegetative propagation is typically an efficient method of maintaining specific traits of plant genetic resources. Considerable amount of superior quality planting materials can be produced through clonal propagation within short time (Baul *et al.,* 2010; Hartmann *et al.,* 2011; Wetzstein *et al.,* 2018). Adventitious rooting, as one of the most important methods of plant vegetative propagation, is a requirement for successful production of practical clones. It is also one of the most efficient methods for fast commercial production of horticultural plants worldwide (Shi-Weng *et al.,* 2009b). Hardwood cuttings are one of the least expensive and easiest methods of vegetative propagation. They are not easily perishable, may be shipped safely over long distances and require little or no special equipment during rooting (Hartmann *et al.,* 2011).

The GF677 (*Prunus amygdalus* × *Prunus persica*) is a valuable rootstock, which has a wide range of compatibility with various cultivars and species (Karimi and Yadollahi, 2012; Gainza *et al.,* 2015). It is tolerant against donor Fe deficiency and suitable for semi-drought conditions, calcareous and poor fertility soils (Legua *et al.,* 2012; Bagheri *et al*., 2016; Ranjbar *et al.,* 2019). Therefore, to find the appropriate methods to improve the GF677 propagation is highly important (Tsipouridis and Thomidis, 2004; Tsipouridis *et al.,* 2005; Karimi and Yadollahi, 2012; Sarikhani *et al.,* 2017).

Environmental and endogenous factors such as temperature, light conditions, plant growth regulators (PGRs), carbohydrate, mineral elements and other molecules, may act as signals and induce groups of cells to alter the result in adventitious rooting (Shi-Weng *et al.,* 2009a; Hartmann *et al.,* 2011). In addition to the genotype of the plant, the nutritional status, the phenological stage, the environmental factors, and the climatic conditions cause seasonal variations in the rooting capability of the woody cuttings (Hartmann *et al.,* 2011). Application of plant growth regulators (PGRs) is one of the most effective procedures to increase root initiation, rooting percentages, and quality and uniformity of roots. The most widely used PGRs for this purpose is indole butyric acid (IBA) (Ozelbaykal and Gezerel, 2005; Asl moshtaghi and Shahsavar, 2011; Nazary and Yadollahi, 2012; Nag *et al.,* 2013; Caplan *et al.,* 2018). Furthermore, it has been reported that polyamines (PAs) are able to promote root formation and root development in difficult-to-root plants; particularly, Put has displayed a better response in comparison with other PAs (Rey et al., 1994; Wu et al., 2010; Sivanandhan et al., 2011; Silvestri et al., 2018). Rey *et al.* (1994) found a powerful enhancing effect of PAs on microshoots rooting of hazelnut. PAs improved rooting when microshoots were treated with IBA in a synergistic mode; probably generated a better induction of roots, while PAs had only a limited beneficial impact on rooting when applied without IBA. Additionally, Put in combination with IBA promoted early rooting and increased the rooting percentage in hazelnut (Cristofori *et al.,* 2010), olive (Rugini *et al*., 2016) and in *Ficus* (Ghasemi and Kosh-Khui, 2019). Some studies demonstrated a possible improvement of the auxin stimulation on adventitious root formation when it is mixed with co-factors such as phenolics (Bartolini and Tattini, 1986; De-Klerk *et al.,* 1999), flavonoids (Lewis *et al.,* 2011) and hydrogen peroxide (H_2O_2) (Sebastiani and Tognetti, 2004; Shi-Weng *et al*., 2009a and b). Moreover, there is a connection between PAs effects on rooting enhancement of cuttings and increase of peroxidase activity at the basal end of cuttings (Rugini *et al.,* 1997).

The relationship between tissue carbohydrate contents and rooting has remained controversial for many years (Tsipouridis and Thomidis, 2004). Most of the tree species can be rooted with leafy cuttings rather than leafless cuttings (Ky-Dembele *et al*., 2011). Indeed, further carbohydrate substances, seasons and cutting date are critically important elements. For instance, some cuttings as quince can root at any season of the year, whereas cuttings obtained from another species such as cherry and olive root are only successful at a certain time of the year (Hartmann and Loreti, 1965; Hartmann *et al.,* 2011). Ucler and Parlak (2004) examined the influence of IBA and cutting date on rooting of semi-hardwood cuttings of kiwifruit and reported that the cuttings which were taken in August had better rooting compared to those taken in July. In addition, they concluded that cutting date had a significant influence on rooting potential.

In this context, the aim of this research was to evaluate the effects of IBA, Put and H_2O_2 in different concentrations and seasons, separately or in combination with each other, on rooting of GF677 rootstock.

MATERIALS AND METHODS

Location and plant material

This experiment was conducted in the research greenhouse of Bu-Ali Sina University, Hamedan, Iran (1741.5 masl, 34°47′N, 48°30′E), as a completely randomized design with three replications and 12 cuttings in each replication. Uniform cuttings of GF677 (*Prunus amygdalus* × *Prunus persica*) were collected from Paradise Nursery in Hamedan province and Sanaz Nursery in Zanjan province. At first, the semi-hardwood cuttings with about 30 cm length were immersed in Put or H_2O_2 solutions for 30 s, and then treated with IBA for 10 s. Subsequently, cuttings were planted diagonally in a medium involving sand and perlite with a ratio of 80 and 20%, respectively. Furthermore, the rooting bed was covered with covering film to maintain the moisture content. The research included three experiments as following:

First experiment

The first experiment was done on July 6th, and semi-hardwood leafy cuttings were taken from the middle third of the current season branches of the 3-year-old GF677 donor plants. The cuttings were treated with Put and H_2O_2 in seven levels including distilled water (control), Put at three concentrations of 800, 1600 and 3200 mg L^{-1} and H_2O_2 at three concentrations of 1.5, 3 and 6 % w/v, as one factor. Another factor was IBA in four concentrations of 0, 1000, 2000 and 3000 mg L^{-1}.

Second experiment

The experiment was prepared on March 6th and hardwood cuttings without leaf were taken from the current season branches of the GF677 donor plants. Similar to the first experiment, the cuttings were treated with IBA, Put and H_2O_2.

Third experiment

This experiment was performed on October 16th and semi-hardwood leafy cuttings were taken from the current season branches of the GF677 donor plants. Based on results of first and second experiments, the desired levels of IBA (0, 1000 and 2000 mg L^{-1}), Put (800 and 1600 mg L^{-1}) and H_2O_2 (1.5 and 3 % w/v) were used at this stage.

In all three experiments, cuttings were taken in early morning. Immediately after treatment, the cuttings were cultured on the medium mentioned previously. The moisture content was monitored daily. After three months, cuttings were removed from the culture medium and some traits were analyzed such as callus proliferation, rooting of cuttings, fresh and dry weight of rooted and/or callused cuttings.

Statistical analysis

All data were normalized using arc-sin transformation method, and later statistically analyzed based on full factorial experiment in a completely randomized design by using the SAS software (version 9.1). Analysis of variance and Duncan's multiple-range tests ($P \leq 0.05$) were performed to assess possible significant differences among treatments.

RESULTS AND DISCUSSION

First experiment

The analysis of variances ($P \leq 0.01$) showed significant effects of IBA, Put and H_2O_2 and their interactions on callus proliferation percentage (Data of analysis of variance not shown). The callus proliferation was from 0.00 to 42.33% of treated cuttings taken in July. The highest percentage of callus proliferation (42.33%) was observed in cuttings treated with IBA 1000 mg L^{-1}, which showed no significant differences with some other treatments. However, the lowest percentage (0.00%) corresponded to Put treated cuttings. Furthermore, no callus proliferations were noticed using 3000 mg L^{-1} IBA+1600 mg L^{-1} Put (Table 1).

The effect of IBA, Put and H_2O_2 and their interactions on callus fresh weight were significant at $P \leq 0.01$. Application of IBA at 1000 mg L^{-1} led to the highest callus fresh weight in comparison with other treatments. The lowest callus fresh weight was obtained by Put at 800 mg L^{-1}, 1600 mg L^{-1}, 3200 mg L^{-1}, and also 3000 mg L^{-1} IBA+1600 mg L^{-1} Put with no significant difference from control (Table 1).

Regarding rooting response, the application of IBA, Put and H_2O_2 and their interactions were significant ($P \leq 0.01$) on rooting percentage. Rooting percentage in this experiment was ranged between 0.00 to 13.83%. The highest rooting percentage (13.83%) was obtained by the application of 2000 mg L^{-1} IBA+ 3% H_2O_2. It did not have a significant difference when the treatments: 3000 mg L^{-1} IBA+3200 mg L^{-1} Put, 3000 mg L^{-1} IBA+1.5% H_2O_2 and

1000 mg L^{-1} IBA+1.5% H_2O_2 were used. The results of this study showed that the effect of cuttings preparation time as well as auxin, Put and H_2O_2 treatments were effective on callus formation and rooting of GF677 cuttings. Auxin has been proven to be an important factor in the rooting of many cuttings. Therefore, the internal auxin level is vital in callus production and rooting of cuttings in various plants. In the case of high levels of endogenous auxin, rooting can easily occur in many cases (Hartmann *et al.,* 2011). In contrast, in cases where external auxin treatment is required, the use of IBA as a commercial auxin to enhance rooting of many plants has been demonstrated (Shiozaki *et al.,* 2013). The lowest rooting percentage was gained in all treatments without IBA such as those of control and different concentrations of Put-H_2O_2 (Table 1).

Table 1- Effect of IBA, Put and H_2O_2 on callus proliferation and rooting of GF677 cuttings taken in July.

IBA concentration (mg L^{-1})	Put-H_2O_2 concentration (mg L^{-1} - %)		Callus proliferation (%)	Callus fresh weight (mg)	Rooting (%)
0		0	14.33 a-f	5.67 f-j	0.00 e
		800	0.00 g	0.00 j	0.00 e
	Put	1600	0.00 g	0.00 j	0.00 e
		3200	0.00 g	0.00 j	0.00 e
		1.5	28.16 a-d	12.03 c-h	0.00 e
	H_2O_2	3	37.33 ab	7.30 d-i	0.00 e
		6	17.00 a-f	3.43 g-j	0.00 e
1000		0	42.33 a	62.43 a	8.33 abc
		800	17.33 a-f	7.00 f-j	0.00 e
	Put	1600	5.50 e-g	0.57 ij	2.83 de
		3200	14.16 b-g	5.00 g-j	5.50 bcd
		1.5	31.16 a-c	14.17 c-h	8.33 abc
	H_2O_2	3	30.83 a-c	6.50 e-i	0.00 e
		6	8.33 c-g	16.57 c-h	0.00 e
2000		0	42.33 a	47.07 ab	5.50 bcd
		800	19.66 a-f	24.73 b-d	8.33 abc
	Put	1600	2.83 fg	1.17 ij	5.66 bcd
		3200	11.50 b-g	27.33 bc	5.66 bcd
		1.5	14.33 a-f	5.10 f-j	0.00 e
	H_2O_2	3	25.83 a-d	33.00 bc	13.83 a
		6	8.33 c-g	13.23 c-h	0.00 e
3000		0	19.83 a-f	27.00 b-d	5.66 bcd
		800	5.66 d-g	5.47 h-j	0.00 e
	Put	1600	0.00 g	0.00 j	5.50 bcd
		3200	14.16 b-g	9.00 e-i	11.50 ab
		1.5	5.66 d-g	21.80 b-f	8.66 abc
	H_2O_2	3	17.00 a-f	23.00 b-e	5.66 cd
		6	19.66 a-e	19.10 c-g	5.50 bcd

IBA=indole butyric acid, Put=putrescine and H_2O_2=hydrogen peroxide.
Means with similar letters within each column are not significantly different according to Duncan's test ($P \leq 0.05$).

The effect of IBA treatment on root length ($P \leq 0.05$) was significant. The amount of Put at 1600 and 3200 mg L^{-1}, and also H_2O_2 at 1.5 and 3% had no significant effects on root length in comparison with IBA in all concentrations. The highest root length (5.1 mm) was observed in cuttings treated with 2000 mg L^{-1} IBA with no significant difference between 1000 mg L^{-1} and 3000 mg L^{-1} IBA (Figure 1).

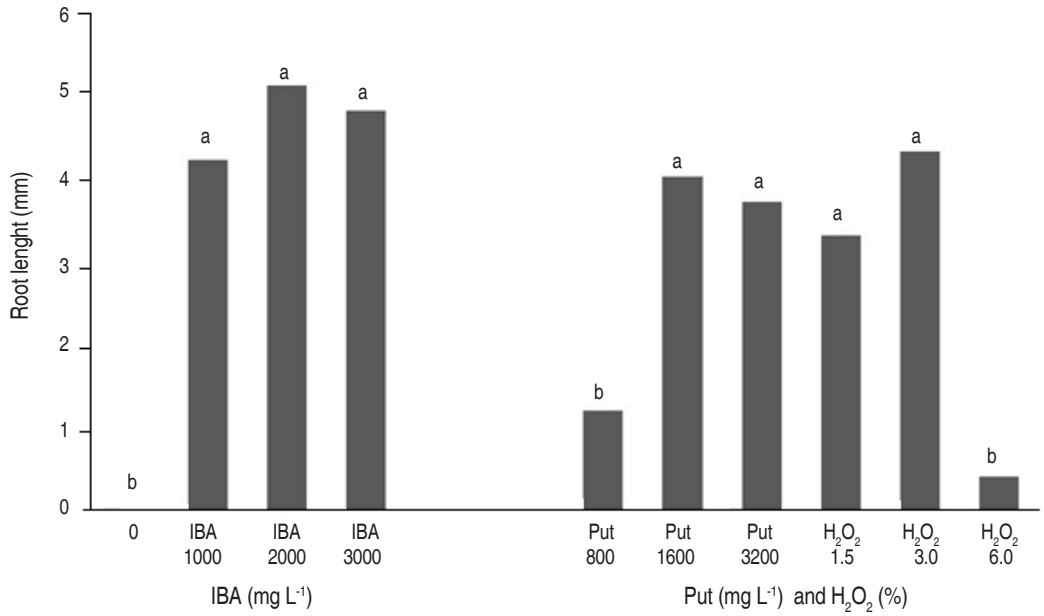

Figure 1. Effect of IBA, Put and H_2O_2 on root length of GF677 cuttings in July. In each group, columns with similar letters have no significant difference according to Duncan's test ($P \leq 0.05$). IBA=indole butyric acid, Put=putrescine and H_2O_2=hydrogen peroxide.

Second experiment

Analysis of variance showed significant effects of IBA, Put and H_2O_2 treatments and their interactions on percentage of callus proliferation ($P \leq 0.01$). The highest callus proliferation was obtained at 1000 mg L^{-1} IBA+1600 mg L^{-1} Put, with no significant difference at 1000 mg L^{-1} IBA+800 mg L^{-1} Put, 1000 mg L^{-1} IBA+3200 mg L^{-1} Put, 1000 mg L^{-1} IBA+ 3% H_2O_2, 2000 mg L^{-1} IBA+800 mg L^{-1} Put and 3000 mg L^{-1} IBA+800 mg L^{-1} Put. The callus proliferation percentages for control and IBA 2000 mg L^{-1} treatments were zero. Also, the IBA at 3000 mg L^{-1} had no significant difference with regard to control (Table 2). These results showed that rooting was gained after callus production. Callus formation of GF677 was coincided with data that was obtained from rooting percentage and number of roots. Researchers have suggested that basal callus formation generally tracks rooting of GF677 with young hardwood trees (Tsipouridis and Thomidis., 2004; Tsipouridis *et al.,* 2005; Karimi and Yadollahi, 2012). Other authors have claimed that callus formation might help the rooting of some plant species with hardwood cuttings (Lodama *et al.,* 2016; Zhou *et al.,* 2018).

Application of IBA, Put and H_2O_2 and their interactions on rooting percentage were significant ($P \leq 0.01$) in cuttings taken in March. The highest rooting percentage (63.88%) was attained from cuttings treated by 2000 mg L^{-1} IBA+3200 mg L^{-1} Put (Figure 2), which did not have a significant difference with the treatments:1000 mg L^{-1} IBA+1600 mg L^{-1} Put (58.32%), 2000 mg L^{-1} IBA+ 800 mg L^{-1} Put (58.31%), 3000 mg L^{-1} IBA+3% H_2O_2 (52.77%), different levels of IBA with 800 mg L^{-1} Put, 2000 mg L^{-1} IBA+3% H_2O_2 (47.21%) (Figure 3), and 3200 mg L^{-1} Put+1000 mg L^{-1} IBA (47.20%). The lowest rooting percentage was resulted from control and treatments without IBA (Table 2).

The application of IBA, Put and H_2O_2 treatments and their interactions on root length were significant at ($P \leq 0.01$). The highest root length was obtained at 2000 mg L^{-1} IBA+1600 mg L^{-1} Put, 1000 mg L^{-1}

IBA+1600 mg L^{-1} Put and 1000 mg L^{-1} IBA+800 mg L^{-1} Put. The root length for control and Put treatments with 1600 mg L^{-1} and 3200 mg L^{-1} was zero and had no significant difference with 800 mg L^{-1} Put, H_2O_2 (1.5 and 3%), IBA (2000 and 3000 mg L^{-1}) treatments (Table 2).

Table 2- The interaction effect of IBA, Put and H_2O_2 on callus proliferation and rooting of GF677 hardwood cuttings in March.

IBA concentration (mg L^{-1})	Put-H_2O_2 concentration (mg L^{-1} - %)		Callus proliferation (%)	Rooting (%)	Root number	Root length (mm)	Root fresh weight (mg)	Root dry weight (mg)
0		0	0.00 j	0.00 g	0.00 l	0.0 f	0.00 n	0.00 k
		800	22.21 g-i	2.77 fg	0.66 jkl	3.3 ef	0.06 n	0.03 jk
	Put	1600	38.88 e-g	0.00 g	0.00 l	0.0 f	0.00 n	0.00 k
		3200	27.77 gh	0.00 g	0.00 l	0.0 f	0.00 n	0.00 k
		1.5	41.65 d-g	2.77 fg	2.33 j-l	10.0 d-f	0.56 mn	0.13 i-k
	H_2O_2	3	49.98 b-f	2.77 fg	0.33 kl	3.3 ef	0.40 mn	0.10 jk
		6	58.31 b-e	8.33 fg	3.33 j	15.0 b-d	3.06 k-m	0.93 f-j
1000		0	24.99 g-i	27.77 de	23.00 d-g	16.6 a-d	12.66 d-h	3.33 b-e
		800	83.31 a	58.31 ab	36.66 a-d	28.3 a	29.80 a	6.16 ab
	Put	1600	83.33 a	58.32 ab	26.00 c-f	28.3 a	27.63 ab	7.16 a
		3200	63.88 a-d	47.20 a-c	31.00 a-d	20.0 a-d	18.80 c-e	4.23 a-d
		1.5	44.43 c-g	30.54 de	17.33 e-i	21.6 a-d	9.40 g-i	2.10 c-f
	H_2O_2	3	69.41 ab	41.65 b-d	18.66 e-h	15.0 b-d	12.00 e-i	2.66 c-f
		6	58.31 b-e	27.77 de	8.66 i	18.3 a-d	4.56 j-l	0.73 f-j
2000		0	0.00 j	5.55 fg	3.00 j	10.0 d-f	1.30 m-n	0.33 g-k
		800	66.64 a-c	58.31 ab	44.66 ab	25.0 a-c	19.56 b-d	4.63 a-c
	Put	1600	55.53 b-e	36.09 c-e	29.00 b-e	28.3 a	14.66 d-g	2.33 c-f
		3200	55.53 b-e	63.88 a	46.33 a	21.6 a-d	26.29 a-c	6.83 a
		1.5	30.54 f-h	27.76 de	15.66 f-i	26.6 ab	8.30 h-j	2.10 d-g
	H_2O_2	3	55.54 b-e	47.21 a-c	34.66 a-d	20.0 a-d	16.90 d-f	3.63 a-d
		6	38.87 e-g	19.43 ef	11.00 hi	13.3 c-e	6.60 i-k	1.43 e-i
3000		0	5.55 ij	5.55 fg	2.00 j-l	10.0 d-f	0.80 mn	0.20 h-k
		800	66.64 a-c	55.54 ab	38.00 a-c	16.6 a-d	14.73 d-g	4.03 a-d
	Put	1600	24.99 g-i	27.76 de	14.66 g-i	15.0 b-d	10.60 f-i	2.50 c-f
		3200	30.55 f-h	30.54 de	38.00 a-c	25.0 a-c	14.66 d-g	3.86 a-d
		1.5	13.88 h-j	19.44 ef	12.00 hi	23.3 a-c	3.93 j-l	1.10 e-h
	H_2O_2	3	55.54 b-e	52.77 ab	33.00 a-d	25.0 a-c	12.43 d-h	2.86 c-e
		6	16.66 h-j	5.55 fg	2.66 jk	13.3 c-e	1.50 l-n	0.26 h-k

IBA=indole butyric acid, Put=putrescine and H_2O_2=hydrogen peroxide.
Means with similar letters within each column have no significant differences based on Duncan's test ($P \leq 0.05$).

Figure 2. GF677 semi-hardwood cuttings three months after treatment of 2000 mg L^{-1} IBA+3200 mg L^{-1} Put in March.

Figure 3. GF677 semi-hardwood cuttings three months after treatment of 2000 mg L^{-1} IBA+3% H_2O_2 in March.

The application of IBA, Put and H_2O_2 treatments and their interactions on root number were highly significant ($P \leq 0.01$). The highest root number (46.3) was observed in 2000 mg L^{-1} IBA+3200 mg L^{-1} Put, 2000 mg L^{-1} IBA+800 mg L^{-1} Put, 3000 mg L^{-1} IBA+800 mg L^{-1} Put, 1000 mg L^{-1} IBA+800 mg L^{-1} Put and 1000 mg L^{-1} IBA+3200 mg L^{-1} Put treatments. The lowest number of roots was recorded for control and 1600 and 3200 mg L^{-1} Put, while they did not have significant differences with 1.5 and 3% H_2O_2 and 3000 mg L^{-1} IBA treatments (Table 2). The study showed that Put had some effects on root formation and its growth. Data on length and dry weight of roots illustrated that application of Put in the rooting of GF677 resulted in better quality roots compared with IBA treated cuttings. Moreover, concentrations of 800 and 1600 mg L^{-1} Put were better than its higher concentration (3200 mg L^{-1}) on the above-mentioned traits. PAs have been showed to increase root elongation and growth by increasing cell division. The amount of Put increases during elongation in the differentiation zone, indicating that PAs are involved in root development (Tang and Newton, 2005). In fact, application of PAs increases the synthesis of internal PAs in plant tissue (Vondrakova *et al.,* 2015). Increasingly, PAs are associated with increased mitotic activation and increased primary and lateral roots. The presence and involvement of genes are also related to the synthesis of Pas, which play a role in root development in the presence of PAs (Mahdavian *et al.,* 2020). Supplementary using of PAs as a contemporary group of plant hormones considerably enhanced the number of rooting saplings of GF677.

This study revealed that Put by-itself did not have much effect on rooting of GF677 cuttings; however, a greatest effect was seen when IBA was used. IBA generates the root induction and Put stimulates the root growth, increasing synergism between both. Similar results also were obtained by Karimi and Yadollahi (2012). They showed that the highest weight of dry roots was attained with 2 mM Put treatments, and the lowest weight of roots was observed with treatment of 3000 mg L^{-1} IBA. The rate of saplings along callus was substantially more exclusive in 2 and 4 mM Put and 1500 mg L^{-1} IBA treatment and the lowest number of the saplings with callus was noticed with a treatment of 3000 mg L^{-1} IBA (Karimi and Yadollahi, 2012).

Third experiment

The application of IBA, Put and H_2O_2 and their interactions on callus proliferation percentage were significant ($P \leq 0.01$). The highest percentage of callus proliferation was observed under the treatments: 800 mg L^{-1} Put (55.55%), 1000 mg L^{-1} IBA+800 mg L^{-1} Put (41.65%), 3% H_2O_2 (36.1%), 1000 mg L^{-1} IBA (30.54%) and 2000 mg L^{-1} IBA (24.99%) (Table 3).

Table 3- The interaction effect of IBA, Put and H_2O_2 on rooting and callus proliferation of GF677 semi hardwood cuttings in October.

IBA concentration (mg L^{-1})	Put-H_2O_2 concentration (mg L^{-1} - %)		Callus proliferation (%)	Root number	Callus fresh weight (mg)	Callus dry weight (mg)	Root fresh weight (mg)	Root dry weight (mg)
0 (control)		0	22.21 c-g	1.66 de	9.95 c	3.52 bc	3.10 cd	0.68 de
	Put	800	55.55 a	6.33 a-d	35.31 b	8.67 b	16.96 ab	5.62 b
		1600	25.00 c-f	1.66 de	29.09 bc	6.07 bc	1.10 cd	0.27 de
	H_2O_2	1.5	27.76 b-e	0.0 e	24.50 bc	5.46 bc	0.00 d	0.00 e
		3	36.10 bc	0.33 e	29.01bc	6.74 bc	0.75 c	0.23 de
1000		0	30.54 b-d	8.33 ab	25.88 bc	4.62 bc	4.45 c	1.44 cd
	Put	800	41.65 b	2.33 c-e	74.60 a	15.25 a	2.25 cd	0.82 de
		1600	19.44 d-g	2.66 c-e	34.80 b	6.39 bc	2.96 cd	0.89 de
	H_2O_2	1.5	8.33 gh	1.00 e	8.21 c	1.63 c	0.65 cd	0.16 e
		3	13.88 e-h	3.33 c-e	16.55bc	3.93 bc	2.58 cd	0.95 de
2000		0	24.99 c-f	9.66 a	31.95 b	5.93 bc	30.38 a	10.99 a
	Put	800	13.88 e-h	3.66 b-e	15.12 bc	2.99 bc	4.63 c	1.51 cd
		1600	11.11 f-h	7.33 a-c	15.88 bc	3.48 bc	15.01 b	3.41 bc
	H_2O_2	1.5	2.77 h	0.0 e	9.51 c	1.83 c	0.00 d	0.00 e
		3	19.44 d-g	3.00 c-e	21.67 bc	4.36 bc	2.08 cd	0.68 de

IBA=indole butyric acid, Put=putrescine and H_2O_2=hydrogen peroxide.
Means with similar letters within each column have no significant differences based on Duncan's test ($P \leq 0.05$).

Data analysis showed significant effects of IBA ($P \leq 0.05$), Put and H_2O_2 ($P \leq 0.01$) and their interactions ($P \leq 0.01$) on callus fresh weight. The highest callus fresh weight was observed at 1000 mg L^{-1} IBA+800 mg L^{-1} Put (74.6 mg) and the lowest amount of callus fresh weight was recorded for control (9.95 mg), 2000 mg L^{-1} IBA+1.5% H_2O_2 (9.51 mg) and 1000 mg L^{-1} IBA+1.5% H_2O_2 (8.21 mg) (Table 3).

The effect of IBA ($P \leq 0.05$), Put and H_2O_2 ($P \leq 0.01$) and their interactions ($P \leq 0.01$) on callus dry weight was significant. Highest callus dry weight (15.25 mg) was obtained in cutting treated by 1000 mg L^{-1} IBA+800 mg L^{-1} Put. Also, the least callus dry weight observed from 2000 mg L^{-1} IBA+1.5% H_2O_2 (1.83 mg), and 1000 mg L^{-1} IBA+1.5% H_2O_2 (1.63 mg), while they did not have a significant difference with control (Table 3).

The IBA treatment had no significant difference with the interaction treatments on rooting percentage. There were no significant differences among different amounts of IBA, Put and H_2O_2 with control. The rooting percentage in 1.5% H_2O_2 treatment (0.92) decreased in comparison with control (Table 4). The activity of the enzymes at the rooting zone of stem cuttings may facilitate easy and rapid cell differentiation toward rooting. Peroxidase is an enzyme that initially increases its activity in root initiation and root development and has a positive effect on rooting. In addition, it is used as a marker in rooting to improve and enhance rooting (Hartmann *et al.,* 2011). H_2O_2 is a co-enzyme that catalyzes the oxidation of a variety of organic compounds. Numerous rooting studies have shown that H_2O_2 plays an essential role in rooting of cuttings (Sebastiani and Tognetti, 2004; Shi-Weng *et al.,* 2009a and b). Shi-Weng *et al.* (2009a) demonstrated that H_2O_2 may act as a signal molecule, involved in the auxin-induced formation of adventitious root formation. Their results showed that higher concentrations of H_2O_2 were required during the induction of adventitious root formation. Moreover, applying of IBA with H_2O_2 significantly refined the rooting of saplings compare to untreated ones; but the rooting percentage was low compared when only IBA was used. According to Sebastiani and Tognetti (2004), IBA+H_2O_2 caused considerable higher root number comparing with only IBA treatment on olive cultivars. The effect of Put and H_2O_2 treatments on root length was significant at ($P \leq 0.01$), while IBA treatment and their interactions were not significant. The highest root length was observed with 800 mg L^{-1} Put treatment (23.4 mm) and the least root length was obtained at 3% H_2O_2 treatment with (4.8 mm) (Table 4).

Table 4- Effect of IBA, Put and H_2O_2 on root length and rooting of GF677 stem cuttings in October.

Treatments		Rooting (%)	Root length (mm)
IBA concentration (mg L^{-1})			
	0 (Control)	4.34 a	9.3 a
	1000	8.26 a	10.4 a
	2000	7.21 a	11.6 a
Put-H_2O_2 concentration (mg L^{-1} - %)			
Put	800	6.47 a	23.4 a
	1600	8.21 a	9.4 abc
H_2O_2	1.5	0.92 b	5.5 c
	3	6.47 a	4.8 bc

IBA=indole butyric acid, Put=putrescine and H_2O_2=hydrogen peroxide. Means with similar letters within each column have no significant differences based on Duncan's test ($P \leq 0.05$).

Analysis of variance showed significant effect of Put and H_2O_2 ($P \leq 0.01$), IBA ($P \leq 0.05$) and their interactions ($P \leq 0.05$) on root number. The Highest root number was obtained at 2000 mg L^{-1} IBA only, 1000 mg L^{-1} IBA only and 2000 mg L^{-1} IBA+1600 mg L^{-1} Put. The least root number resulted under treatments of: 1.5 and 3% H_2O_2, 1000 mg L^{-1} IBA+1.5% H_2O_2 and 2000 mg L^{-1} IBA+1.5% H_2O_2, which had no difference with control, 1600 mg L^{-1} Put, 1000 mg L^{-1} IBA+800 mg L^{-1} Put, 1000 mg L^{-1} IBA+1600 mg L^{-1} Put, 1000 mg L^{-1} IBA+3% H_2O_2, 2000 mg L^{-1} IBA+800 mg L^{-1} Put and 2000 mg L^{-1} IBA+3% H_2O_2 treatments (Table 3).

The application of IBA, Put and H_2O_2 treatments and their interactions on fresh and dry weight of roots were highly significant ($P \leq 0.01$). The most root fresh weight (30.38 mg) was observed by 2000 mg L^{-1} IBA treatment, whereas the least root fresh weight was obtained using the treatments: 1.5% H_2O_2 (0 mg), 2000 mg L^{-1} IBA+ 1.5% H_2O_2 (0 mg), 1600 mg L^{-1} Put (1.1 mg), 1000 mg L^{-1}

IBA+1.5% H_2O_2 (0.65 mg) and 3% H_2O_2 (0.75 mg) (Table 3). The effects of IBA, Put and H_2O_2 treatments and their interactions on root dry weight were significant at ($P \leq 0.01$). The highest root dry weight resulted using 2000 mg L^{-1} IBA. Moreover, root dry weight in 1.5% H_2O_2 and 2000 mg L^{-1} IBA+1.5% H_2O_2 were zero, which had no significant difference with control and other treatments (Table 3). In the present study, the use of the only auxin (IBA) produced callus and rooting in the three times of cutting collection. The need of using auxin for rooting of GF677 cuttings has been reported in previous studies (Tsipouridis and Thomidis. 2004; Tsipouridis *et al.,* 2005; Karimi and Yadollahi, 2012). Based on Tsipouridis *et al.* (2005) the appropriate concentration is between 500 and 2500 mg L^{-1} IBA. Similar results were obtained by Karimi and Yadollahi (2012) who reported rooting of 57% of GF677 cuttings when treated by 1500 mg L^{-1} IBA using a quick-dip method. The rooting decreased at 3000 mg L^{-1} IBA. High concentrations of IBA may have toxic effects on cuttings, thereby reducing the rooting (Karimi and Yadollahi, 2012). In addition, in this study, it was observed that time of cuttings preparation was very effective on callus formation and rooting of GF677 cuttings. Little rooting was observed in cuttings prepared in July and October. In contrast, rooting was much higher in those cuttings that were taken in March. The rooting success of peach cuttings has been reported to be affected by the date of collection of cuttings (Tworkoski and Takeda, 2007). The effect of cuttings collection date on the rooting of many cuttings, including olives (Hartmann and Loreti, 1965; Khajehpour *et al.,* 2014) and peach (Tofanelli *et al.,* 2003), has been demonstrated. It seems that several factors related to the time of preparation of cuttings can be effective on rooting of cuttings (Hartmann *et al.,* 2011). Khajehpour *et al.* (2014) observed no rooting on the cuttings collected in October, but the cuttings collected in August rooted well. They also reported better rooting in cuttings taken in March compared to those taken in late summer or early autumn (Khajehpour *et al.,* 2014). However, conflicting results have been reported by other researchers. Tofanelli *et al.* (2003) for peach cuttings reported that the most favorable time for collecting of cuttings is between October 25 and November 13.

CONCLUSION

The best results of callus formation and rooting were observed in cuttings prepared in March. The current experiment showed that the application of IBA, Put and H_2O_2 in comparison with control, increased the rooting of hard and semi-hardwood cuttings of GF677 rootstock. Treatment of cuttings by only IBA was more effective than only Put or H_2O_2. Additionally, IBA at concentrations of 1000 and 2000 mg L^{-1} indicated better influence on rooting. Also, the use of inexpensive chemicals such as Put or H_2O_2 together with IBA had a positive effect on increasing the rooting of GF677 cuttings, and the use of Put had a greater effect on root growth. The high concentration of only Put at 3200 mg L^{-1} and H_2O_2 at 6% caused the least effect on measured traits while these dosages combined with IBA showed a favorable effect on mentioned factors.

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Kinetic study and modeling of xylitol production using *Candida tropicalis* in different culture media using unstructured models

UNIVERSIDAD NACIONAL DE COLOMBIA

Estudio cinético y modelado de la producción de xilitol utilizando *Candida tropicalis* en diferentes medios de cultivo mediante modelos no estructurados

https://doi.org/10.15446/rfnam.v74n2.92270

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ABSTRACT

Unstructured models for cell growth, xylose consumption and xylitol production were applied for to describe the fermentation kinetics of xylitol production using *Candida tropicalis* in synthetic medium, and in non-detoxified oil palm empty fruit bunch (OPEFB) hydrolysate at 100 mL flask scale. In synthetic medium, the experimental maximum specific growth rate (μ_{max}) and the cell mass yield factor ($Y_{X/S}$) were closer to the results of the Tessier model than those of the Contois and Monod models. Whereas, in non-detoxified OPEFB hydrolyzate, these parameters were closer to the results of the Contois model than those of the Tessier and Monod models. According to the models' results, xylitol is mainly produced during the cell growth phase. The Tessier model in synthetic medium and Contois model in non-detoxified OPEFB hydrolysate had a coefficient of variation in growth kinetics of 32 and 33%, respectively. The significance of this study lies in simplifying the fermentation process through a an unstructured and non-segregated model using three events at the same time, cell growth, substrate consumption and metabolite production.

Keywords:
Fermentation
Oil palm empty fruit bunch
Unstructured models
Xylose

RESUMEN

Se aplicaron modelos no estructurados de crecimiento celular, consumo de xilosa y producción de xilitol para describir la cinética de fermentación en la producción de xilitol usando *Candida tropicalis* en medio sintético y en hidrolizado de raquis de la palma de aceite no desintoxicado (OPEFB siglas en inglés) en escala de matraz de 100 mL. En medio sintético, la tasa máxima de crecimiento específico experimental (μ_{max}) y el factor de rendimiento de masa celular ($Y_{X/S}$) estuvieron más cerca de los resultados del modelo Tessier que los de los modelos Contois y Monod. Mientras que, en el hidrolizado de OPEFB no desintoxificado, estos parámetros se acercaron más a los resultados del modelo Contois que a los de los modelos Tessier y Monod. Según los resultados de los modelos, el xilitol se produce principalmente durante la fase de crecimiento celular. El modelo de Tessier en medio sintético y el modelo de Contois en hidrolizado de OPEFB no desintoxificado tuvieron un coeficiente de variación en la cinética de crecimiento de 32 y 33%, respectivamente. La importancia de este estudio radica en simplificar el proceso de fermentación mediante un modelo no estructurado y no segregado utilizando tres eventos al mismo tiempo, crecimiento celular, consumo de sustrato y producción del metabolito.

Palabras clave:
Fermentación
Racimo vacío de palma de aceite
Modelos no estructurados
Xilosa.

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Received: February 15, 2021; Accepted: April 07, 2021
Rev. Fac. Nac. Agron. Medellín 74(2): 9583-9592. 2021
ISSN 0304-2847 / e-ISSN 2248-7026

Hemicellulose, the second most abundant polysaccharide in nature is used to generate value-added products such as xylitol, widely used in the food industry, as well as in products for oral hygiene, pharmaceuticals and cosmetics (Prakasham *et al*., 2009) . The use of lignocellulosic material such as oil palm empty fruit bunch (OPEFB) is an alternative for the production of this sugar alcohol. Colombia is the largest producer of oil palm in Latin America and the fourth largest producer in the world (Fedepalma, 2014). The OPEFB constitutes 21% of the total weight of harvested fruits and is one of the most promising resources of biomass waste, because they can be used as a substrate for the production of different compounds with chemical and biochemical processes, such as xylitol (Manjarres-Pinzon *et al*., 2017). During the hydrolysis, the polymers present in the hemicellulosic fraction are broken until obtaining xylose, a sugar that is converted to xylitol by fermentation. Xylitol yield and substrate consumption must be high and the production costs low for the xylitol industrial application (Yewale *et al*., 2017). In the market, xylitol is produced chemically based on a catalytic reduction of pure xylose, which is generally obtained from lignocellulosic residues, under high pressure and temperature using expensive catalyst (usually Ni-catalyst) (Rao *et al*., 2016). Currently, the commercial production of xylitol from lignocellulosic wastes is not made by biotechnological route; however, biotechnological production of xylose to xylitol offers a better alternative in terms of energy intensity of the production process of xylitol and overall process cost.

Mathematical modeling is an important tool to simulate and predict the operating conditions of a process. The mathematical model is important to deduce if there is inhibition by substrate or by production of a metabolite product, and to show the metabolic behavior of a strain; therefore, in the case of the biotechnological production of xylitol, the mathematical model can indicate the phase of growth where occurs more metabolite production, and what is the behavior at the numerical level to be able to predict results at the experimental level with different fermentation conditions. However, it is necessary to properly understand the biochemical principles of a biotechnological process to build a suitable model (Aguiar *et al*., 2002). It is important to qualitatively describe the system where the problem is evidenced to develop a mathematical model from flask-scale experimental data showing yeast growth, xylose consumption and xylitol production. Furthermore, the fundamental laws that govern the system, the aim of the model, the specification of input and output variables, the accuracy degree, the application scope and the factors during the fermentation process that support the model must be taken into account.

There is few information on modeling the kinetics of xylitol production. Moreover, it has focused on growth prediction of *C. guilliermondii* using non-linear methods (Aguiar *et al*., 2002), as well as on the development of a model with *C. parapsilosis* subjected to limited oxygen conditions (Aranda-Barradas *et al*., 2000) of a growth model of *C. mogii* with the presence of glucose as a co-substrate to facilitate xylitol production (Tochampa *et al*., 2005) and of a *C. tropicalis* growth model, based on the initial concentration of xylose and oxygen that is related to the agitation rate (Mohamad *et al*., 2016).

The diversity of raw materials and the complexity of fermentation processes mean that there are many ways to approach the mathematical description of these processes. There are two main types of mathematical models of growth kinetics: structured and unstructured. Structured models take into account the cell basic aspects and its chemical species in question. Conversely, unstructured models consider microorganisms and/or cells have a simple and fixed composition, and ignores changes in the culture medium resulting from the cell mass concentration (Esener *et al*., 1983). Moreover, models can also be classified into segregated and non-segregated type models. The segregated models refer to a heterogeneous microorganisms population, where different entities with dissimilar ages, shapes, sizes and internal compositions are distinguished in the population; whereas, the non-segregated models consider the cellular behavior is close to that of a single average cell that allows fixing the performance of certain variables during fermentation (Ghosh *et al*., 2012; Kucharska *et al*., 2018).

If complex such as inhibition are excluded, the functions that describe the growth rate of microorganisms can be classified into two main types, depending on whether they involve only the resource concentration in the

medium containing the culture, as in the case of the Monod model, or substrate and biomass densities as in the case of the Contois or Tessier model (Krichen *et al.*, 2017). Monod's equation is applied to the substrates degradation at the concentration that partially saturates the microbial cells activity. The Contois model assumes that the half saturation rate depends on the biomass concentration, while the Tessier model is based on an exponential function (Sakthipriya *et al.*, 2018).

At present, there are no studies showing models of cell growth and xylitol production with OPEFB. Monod, Contois and Tessier models in synthetic media have been adjusted to the growth of yeasts of the genus Candida (Aguiar *et al.*, 2002). The aim of this study was to propose an unstructured and non-segregated model that adequately describes cell growth, xylose consumption and xylitol production in both synthetic medium and in non-detoxified OPEFB hydrolysate at flask scale using *C. tropicalis*.

MATERIALS AND METHODSE

Microorganism and culture medium

The *Candida tropicalis* (ATCC 96745) strain was stored at 4 °C in agar of yeast extract, peptone and xylose (YPX) with a concentration of 20 g L^{-1} of each compound. The strain was subcultured in the same agar at 30 °C for 48 h before carrying out the pre-inoculum. Final pH was 5.6 and the fermentation medium was non-detoxified OPEFB hydrolysate (Manjarres-Pinzon *et al.*, 2017).

Pre-inoculum and fermentation conditions

The pre-inoculum was performed in 100 mL Erlenmeyer flasks with 40 mL of medium and 20 g L^{-1} of xylose substrate. They were incubated at 30 °C and 120 rpm for 18 h. At the end, the Erlenmeyer flasks were inoculated with non-detoxified OPEFB hydrolysate as a fermentation medium aiming at studying their kinetics and modeling their behavior as well. The fermentations were also carried out in 100 mL Erlenmeyer flasks with 40 mL of YPX synthetic medium. The inoculum concentration was 1.6 g L^{-1}, the ratio of medium volume and Erlenmeyer flask volume was 0.4, pH 5.6 and 120 rpm of agitation rate. For the kinetic study, the fermentations with non-detoxified OPEFB hydrolysates were performed for 96 h.

Analytical methods

Cellular concentration was determined by optical density spectrophotometry at 620 nm (Genesys 20, Thermo Scientific, Waltham, MA) and correlated with the dry weight method (Manjarres *et al.*, 2018). Xylose and xylitol quantification was performed using an HPLC system (Shimadzu Prominence, Canby, OR), with IR detector, equipped with an Aminex HPX-87H column (Biorad). Elution was carried out with aqueous H_2SO_4 (0.005 M) at a flow rate of 0.6 mL min^{-1}. The oven temperature was maintained at 65 °C. The injection volume was 20 µL (Manjarres-Pinzon *et al.*, 2017). Samples were prepared in duplicate and filtered.

Fermentation models

Cell growth, substrate consumption and xylitol production kinetics were studied in flask scale with 100 mL capacity. The models studied for cell growth were Monod (Equation 1), Contois (Equation 2) and Tessier (Equation 3); Luedeking-Piret model (Equation 4) was applied for xylitol production and the general mass balance equation of substrate consumption was used for xylose consumption (Equation 5) (Aguiar *et al.*, 2002).

$$\frac{dX}{dt} = \frac{\mu_{max} XS}{K_s + S} \quad (1)$$

$$\frac{dX}{dt} = \frac{\mu_{max} XS}{BX + S} \quad (2)$$

$$\frac{dX}{dt} = \mu_{max} XS\left(1 - e^{\left(\frac{-S}{K_t}\right)}\right) \quad (3)$$

$$\frac{dP}{dt} = \alpha\frac{dx}{dt} + \beta X \quad (4)$$

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}}\frac{DX}{dt} - msX - \frac{1}{Y_{P/S}}\frac{dP}{dt} \quad (5)$$

Where, the estimated parameters were μ_{max}, B, α, β, $Y_{X/S}$ and $Y_{P/S}$, K_S and K_t, according to the model used. The experimental maximum specific growth rate (μ_{max}) was calculated from the slope of the regression line of the natural logarithm of the cell numbers (LnX) in the increasing phase as a time function (Mohamad *et al.*, 2016). $Y_{X/S}$ is the biomass yield based on substrate consumption and it was considered that the entire substrate was used for cell growth. K_S is the saturation

or substrate utilization constant, numerically equal to the substrate concentration where $\mu=\mu_{max}/2$. In the Tessier model, growth inhibition by substrate depletion is included, thus, μ is nullified or tends to zero with low substrate concentrations. K_t is the substrate growth inhibition constant.

The exponential phase, where xylitol is produced, and the stationary phase were used for modeling. The substrate consumption was oriented to cell growth. In a batch system, the change in cell concentration depends solely on cell growth. The cell can consume the substrate in three ways: cell growth, cell maintenance and metabolite production. In the substrate equation (Equation 5), ms is the cellular maintenance proportional to the biomass present, $Y_{X/S}^{-1}$ is the substrate fraction used for cell growth and $Y_{P/S}^{-1}$ is the substrate fraction used to produce xylitol. In the Luedeking-Piret model (Equation 4), the first term is oriented to the ability of the cell during cell growth to produce the metabolite (growth or exponential phase); whereas, the second term indicates the metabolite production based on the biomass present in the environment (cellular maintenance or stationary phase). If the product is obtained mainly during cell growth, β must have a low value, and α a high value. Primary metabolites are produced in the growth phase, where xylitol is one of them; on the contrary, secondary metabolites are generally obtained in the stationary phase.

Unstructured kinetic model

Experimental data for kinetic parameters estimation were used. The modeling protocol consists of defining a mathematical model, making a numerical solution scheme, applying numerical solution methods and checking the numerical stability of the model. The latter implies that the model represents the real values of a process, or that the solution be consistent and real. The aim of the modeling protocol is to find a routine that is stable and consistent when applied in solving the differential equations that represent the phenomenology under study.

The parameter estimation process was carried out using the software Berkeley Madonna 8.0, where the parameters of both cell growth models, substrate consumption and xylitol production models must be introduced. The software Berkeley Madonna 8.0 implies a numerical solution scheme, generates alerts and makes the modeling protocol as well. After the model numerical stability is done, the study can start. Furthermore, this software has an internal routine that allows calibrating the model and its constants. Model calibration consists of selecting all the variables that are coupled and adjusting the numerical solution that this software gives to the observed data, in order to find the most suitable magnitudes of the constants included in the model. The software, based on the root mean square error (RMSE) and the coefficient of variation (CV), generates a comparison between the observed and estimated data, also begins to generate random values of all the variables, makes iterations and evaluations of RMSE and CV until gets the combination of factors that results in the lowest CV.

The estimation procedure involved the variation of different parameter values for minimizing the differences between the experimental or observed data and those predicted by the model. The applied numerical solution method was Runge-Kutta of order 4 (RK4), which takes the average of 4 slopes and that is the value used to perform the iteration. Parameters initial data were chosen according to the data reported in the literature for this type of fermentation. For instance, the maximum specific growth rate of yeast was taken with values in the range of 0.2-1 h and 0.5-1 h, yields between 0 and 1, parameter B, K_S and K_t were 1.88, 599 and 305, respectively (Aguiar *et al.*, 2002).

RESULTS AND DISCUSSION

Cell growth kinetics

Biomass concentration with respect to fermentation time in YPX synthetic medium and in non-detoxified OPEFB hydrolysate is featured in Figure 1. It is important to note that fermentation in synthetic medium took 33 h, while in non-detoxified OPEFB hydrolysate took 96 h given that this medium contained different inhibitors and nutrients, which prolonged the yeast adaptation phase. Furthermore, no tests were made between 7 to 25 h since in initial experiments, *Candida tropicalis* reached the exponential phase in the YPX synthetic medium at that time. Thus, the aim was to evaluate the different growth phases of this yeast in the medium, and to try to model the fermentation results. The Contois model adequately predicts the experimental values of cell growth in synthetic

medium. However, the behavior of all models predicts cell growth in the first 24 h in non-detoxified OPEFB hydrolysate and after that time, the values of the models tend to be lower than the experimental ones. The considerations that would improve the prediction of the model could be: more precise methods for the cell growth determination such as colony forming unit (CFU) and adjust only two variables for the model instead of three variables (biomass, substrate and product), as was done in this study. Therefore, the type of culture medium was a factor that affected the parameters sensitivity of a kinetic growth model.

In another study, the application of the Contois model in the cell growth of *C. guilliermondii* using a synthetic medium was suitable with small deviations (Aguiar *et al*., 2002). On the other hand, the non-detoxified OPEFB hydrolysate, outside of xylose, has other sugars such as glucose that can contribute to the increase in biomass production. In addition, the non-detoxified OPEFB hydrolysate was supplemented with different salts, such as 4 g L^{-1} yeast extract, 3 g L^{-1} $(NH_4)2SO_4$, 0.5 g L^{-1} $MgSO_4$ $7H_2O$ and 0.1 g L^{-1} $CaCl_2$ $2H_2O$, which help to improve the Candida metabolism (Manjarres-Pinzon *et al*., 2016).

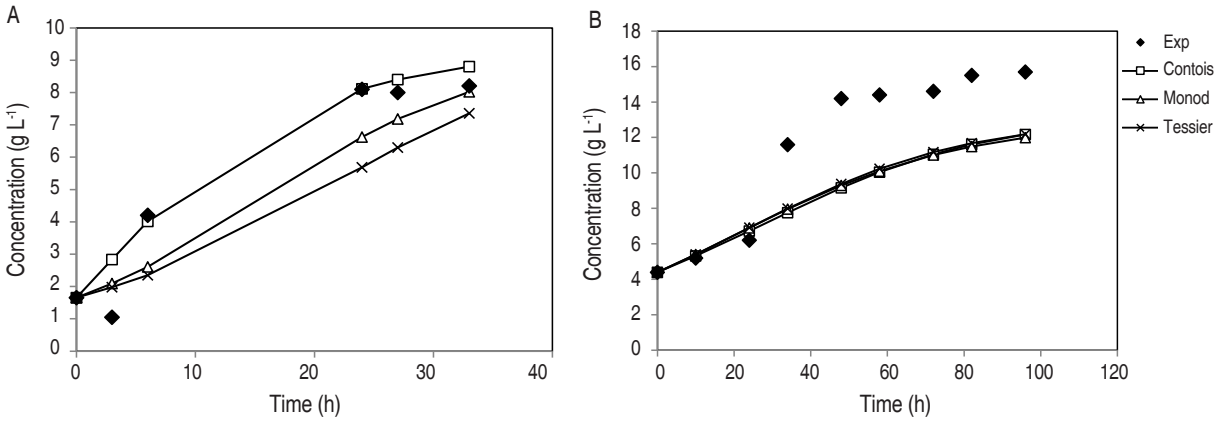

Figure 1. Cell mass concentration during fermentation in synthetic medium YPX (A) and non-detoxified OPEFB hydrolysate (B).

Xylose consumption kinetics

Xylose consumption during fermentation in synthetic medium and in non-detoxified OPEFB hydrolysate is depicted in Figure 2. Xylose concentration gradually decreased over time in both media. Xylose was consumed by 50% in YPX and 70% in non-detoxified OPEFB hydrolysate according to experimental data. Xylitol concentration increased mainly during the rapid xylose consumption period, which confirmed xylitol accumulation due to imbalance factors of the enzymatic activities that involved xylose consumption in the cells (Mohamad *et al*., 2016).

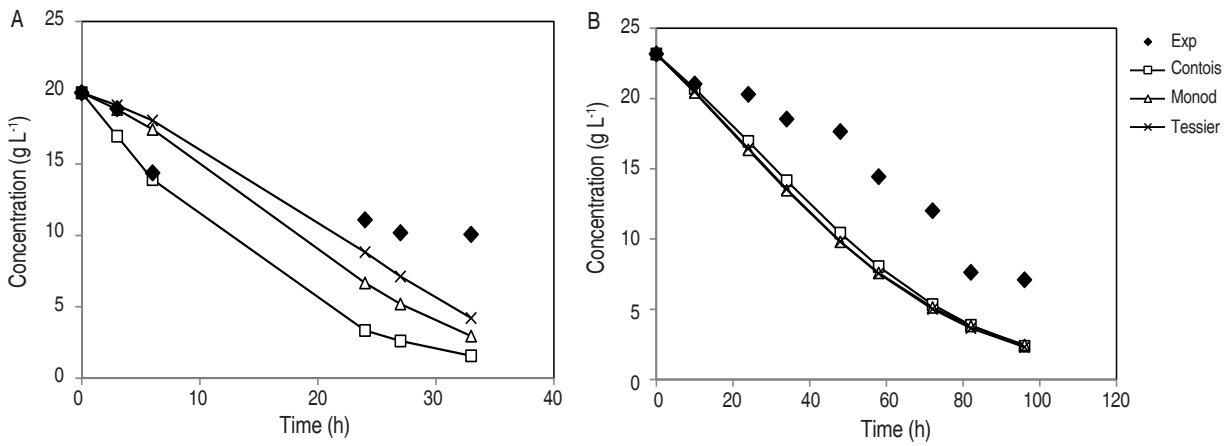

Figure 2. Xylose concentration during fermentation in synthetic medium YPX (A) and non-detoxified OPEFB hydrolysate (B).

In synthetic media, both Monod and Tessier models adequately predicted experimental values in the first 3 h of fermentation; but after 24 h, the models distanced themselves from the experimental values, being the Tessier model the closest in terms of the reported values. The values estimated by the models behave similarly in non-detoxified OPEFB hydrolysate, where it can be emphasized that the estimated results were similar between the models and completely away from the experimental ones. It is necessary to highlight that the modeling of substrate consumption was based on Equation 5, which is closely related to the growth kinetics variables of each model studied.

Xylitol production kinetics

Xylitol production during fermentation in synthetic medium and in non-detoxified OPEFB hydrolysate is shown in Figure 3. All the models were similar to each other and adequately predicted the xylitol production, especially in synthetic medium. However, it should be kept in mind that the base equation in all models for the development of this kinetic was Luedeking-Piret equation, although they also depend on other parameters, which are found both in cell growth and in substrate consumption. The Luedeking-Piret equation is a function of cell growth and cell maintenance to describe product formation (Veeravalli and Mathews, 2018). The Luedeking-Piret equation was originally developed to explain the fermentation kinetics of glucose to lactic acid secretion based on instantaneous growth of bacterial rate and to the bacterial density. Nonetheless, it has been modified to relate the production rate of any metabolite with substrate consumption rate and substrate concentration (Mahdinia *et al.*, 2019). Xylitol concentration

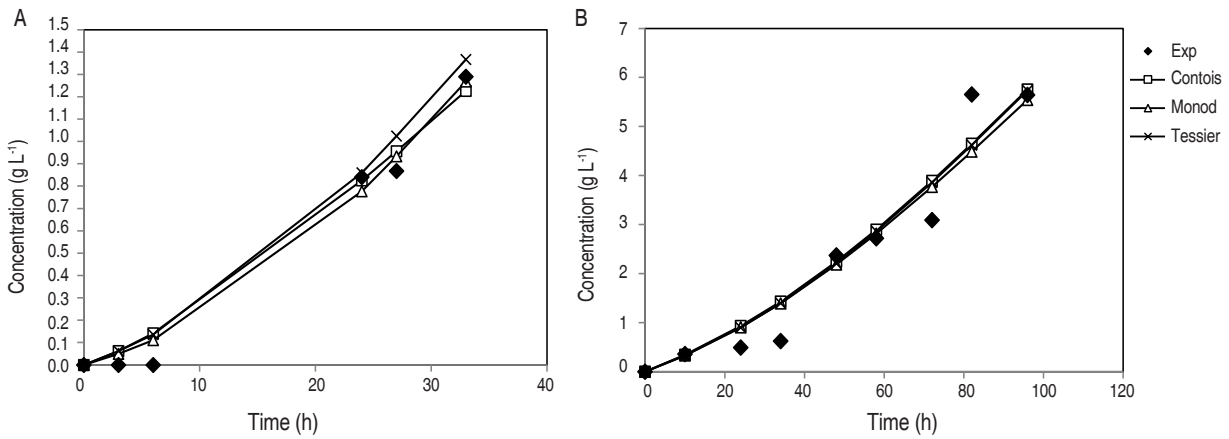

Figure 3: Concentration of xylitol during fermentation in synthetic medium YPX (A) and non-detoxified OPEFB hydrolysate (B).

was higher in non-detoxified OPEFB hydrolysate with a value around 6 g L^{-1} compared to the synthetic medium that was 1.3 g L^{-1}. Studies have shown that xylitol production is affected by many factors such as the type of microorganism, the medium composition, such as initial xylose concentration, inhibitors, etc., and the environmental conditions such as pH, temperature, agitation and aeration (Mohamad *et al.*, 2016; Pappu and Gummadi, 2016).

Model comparison

A kinetic model is helpful to understand and optimize a fermentation process (Zhu *et al.*, 2016). Table 1 shows the estimated parameters of each model during fermentation of *C. tropicalis* in two culture media. Mathematical modeling of cell growth behavior is highly investigated to control, predict and design the processing, stability and safety of food and bioproducts (Guo *et al.*, 2018; Pappu and Gummadi, 2016). The equations of Monod and Tessier are normally used to describe growth with a low cell population; while the Contois model usually works better in cultures with high cell density (Aguiar *et al.*, 2002).

Monod kinetics, expressed as substrate degradation resulting from microbial uptake and cell concentration, has a great effect on degradation rates. Contois kinetics considers the fact that a high concentration of cells adhered to the surface of the particles could inhibit

substrate degradation. Tessier kinetics takes into account the maintenance energy for cellular activity, which means that the maximum growth rate will be reduced when the substrate concentration is lower and microorganisms compete for food sources (Wang and Witarsa, 2016).

Table 1. Estimated values of the parameters of cell growth, xylose consumption and xylitol production during *Candida tropicalis* fermentation in different culture media using unstructured models.

Culture media	Model	μ_{max} [a]	$Y_{X/S}$ [c]	$Y_{P/S}$ [b]	a[e]	b[e]	ms[d]
Synthetic medium YPX	Experimental	0.076	0.65	0.13			
	Monod	0.5	0.7	0.8	0.05	0.004	2.39e -07
	Contois	0.5	0.69	0.8	0.05	0.004	0.03
	Tessier	0.08	0.7	0.8	0.08	0.006	0.089
Non-detoxified OPEFB hydrolysate	Experimental	0.033	0.727	0.364			
	Monod	0.58	0.8	0.7	0.01	0.006	0.051
	Contois	0.026	0.8	0.7	0.02	0.0065	0.000000117
	Tessier	0.1	0.79	0.69	0.001	0.006	0.000000245

[a] Maximum specific growth rate (h^{-1}), [b] Product yield factor (g xylitol g^{-1} xylose), [c] Cell mass yield factor (g cells g^{-1} xylose), [d] Maintenance coefficient (g xylose g^{-1} cells h^{-1}), [e] Constants of the Luedeking – Piret model.

It is necessary to highlight that the experimental value of μ_{max} in the synthetic medium was similar to that reported by the Tessier model, whereas it was closer to the Contois equation in non-detoxified OPEFB hydrolysate (Table 1). The experimental value of μ_{max} was higher in the synthetic medium than in the non-detoxified OPEFB hydrolysate, which indicates that the medium influences the cell growth potential. Generally, yeast extract provides good growth factors and organic nitrogen for microorganisms. In addition, the activities of enzymes involved in cell growth are inhibited by changes in the culture medium (Zhu *et al*., 2016).

The μ_{max} of yeast is usually between 0.2 and 0.5 h^{-1}. In *C. guilliermondii* growth using a synthetic medium for the production of xylitol, a value of μ_{max} was found for the Contois model of 0.25 h^{-1} (Aguiar *et al*., 2002). Nevertheless, in other works, the value has been below than what was previously reported. In xylitol production with *C. parapsilosis* in synthetic medium, μ_{max} was between 0.1 to 0.13 h^{-1} (Aranda-Barradas *et al*., 2000); value of 0.12 h^{-1} was reported with *C. tropicalis* in synthetic medium (Mohamad *et al*., 2016) and with *Debaryomyces nepalensis* in synthetic medium were values from 0.029 to 0.078 h^{-1} (Pappu and Gummadi, 2016). The values of this latter study were close to the experimental values reported in the present study. This indicates that μ_{max} experimentally depends on the fermentation conditions and the microorganisms used as well.

Experimental values of xylitol yield ($Y_{P/S}$) and cellular yield ($Y_{X/S}$) were higher in the non-detoxified OPEFB hydrolysate than in the synthetic medium. Data of the models were closer to the experimental results of $Y_{X/S}$ than of $Y_{P/S}$; the latter being similar among the models. This is probably due to the fact that the base equation used for product modeling was the same for all models as mentioned above. $Y_{X/S}$ and $Y_{P/S}$ values were found between 0.09 to 0.15 (g g^{-1}) and between 0.13 to 0.41 (g g^{-1}), respectively, for xylitol production with *C. tropicalis* in synthetic medium (Mohamad *et al*., 2016). Regarding the xylitol production with *D. nepalensis* in synthetic medium, $Y_{X/S}$ and $Y_{P/S}$ values were reported between 0.1 to 0.6 (g g^{-1}) and 0.1 to 0.5 (g g^{-1}), respectively (Pappu and Gummadi, 2016). The experimental values of the present study were slightly higher for $Y_{X/S}$, whereas the $Y_{P/S}$ data were in the literature range.

Non-growing or slowly growing microbial cultures can recycle much of the metabolic energy derived from initial fermentation of a polysaccharide to boost the additional production of a secondary metabolite (Singh *et al*., 2019). In order to determine if this could be happening in

this study, the amount of substrate used for biomass in the non-growth phase and the maintenance coefficient (ms) for *C. tropicalis* in synthetic medium and in non-detoxified OPEFB hydrolysate were determined (Table 1). The ms indicates the portion of substrate consumed for the maintenance of cellular function and is used as a correction for the microbial growth kinetics (Wang and Witarsa, 2016). In synthetic media, the estimated values of the ms showed that in the Tessier model more xylose was used for its non-growing components, which contributed to xylitol formation; while in the non-detoxified OPEFB hydrolysate, the Monod model showed greater use of xylose for its non-growing components. The estimated values of the ms were low compared to the literature (Pappu and Gummadi, 2016; Singh *et al*., 2019), which could assume that xylitol is mainly produced during the cell growth phase.

The estimated product formation coefficient (α), which represents xylitol production associated with growth, was always higher in all models in both media, except for the Tessier model in non-detoxified OPEFB hydrolysate. This indicates that xylitol formation depends on cell growth. The xylitol production in the stationary phase is represented by β, which is the coefficient of non-growth product. A comparatively low β value suggests that xylitol production remains low in non-growing conditions (Ghosh *et al*., 2012). These results agreed with what was previously reported for the ms.

Statistical criteria to validate fit of data to models, CV and RSME of the models are portrayed in Table 2. CV and RSME were lower in synthetic medium than in non-detoxified OPEFB hydrolysate, except for CV and RSME of Contois model substrate that reported a value of 48% and 5.684, respectively. Lower CV and RSME values represent the best fit of the model (Pappu and Gummadi, 2016). If we focus only on these values, the Monod model would be the most representative in synthetic medium and the Contois model in the non-detoxified OPEFB hydrolysate. Nonetheless, taking into account the experimental values of μ_{max} and $Y_{X/S}$, the models that are closest to reality were Tessier in synthetic medium and Contois in non-detoxified OPEFB hydrolysate. The latter is essential to clarify, since a model is the mathematical representation of a phenomenon or process, and apparently, it can have a good predictive capacity represented in different statistical comparisons and experimental errors; but first of all, the model must be in accordance with the adequate prediction of what happens in reality. The Tessier model in synthetic medium and Contois in non-detoxified OPEFB hydrolysate had a CV in the growth kinetics of 32 and 33%, respectively. This means that the kinetic behavior of the experimental values can be predicted in the conditions posed in this study by around 67%. This may be due to the way the cell concentration was determined and the different assumptions used to apply the models.

Table 2. Estimated parameters and deviations of the models applied in the *Candida tropicalis* fermentation in different culture media.

Culture media	Model	K_s	B	K_t	CV (%)			RSME		
					cell mass	xylose	xylitol	cell mass	xylose	xylitol
Synthetic medium YPX	Monod	104.13	-	-	21	32	12	1.04	4.174	0.062
	Contois	-	15	-	15	48	15	0.788	5.684	0.078
	Tessier	-	-	14.47	32	24	17	1.51	3.223	0.094
Non-detoxified OPEFB hydrolysate	Monod	600	-	-	33	38	23	3.318	5.093	0.54
	Contois	-	1.76	-	33	35	22	3.325	4.769	0.533
	Tessier	-	-	96.24	32	38	22	3.21	5.124	0.525

Since fermentation is a complex bioprocess, it is difficult to obtain a complete profile to reveal what really happens during the entire process and predict the cell growth kinetics and metabolism under certain conditions. Although a huge amount of research on mathematical modeling of microbial processes has been carried out,

the applicability of biological concepts into the models remained limited (Tian *et al*., 2018). It is necessary to keep in mind that these types of processes are complex and depend on various variables.

CONCLUSION

Unstructured models for cell growth, xylose consumption and xylitol production were applied in fermentation by *C. tropicalis* in synthetic medium and in non-detoxified OPEFB hydrolysate. According to the experimental values and the evaluation of model performances, the Tessier model predicted better results in a synthetic environment and the Contois model had a better fit in non-detoxified OPEFB hydrolysate. However, the prediction of these models had an estimate of 67% which coincided with the assumed value with the experimental growth kinetics. Xylitol was a metabolite associated with cell growth.

ACKNOWLEDGMENTS

The authors would like to thank Minciencias-Colombia for providing the Katherine Manjarres-Pinzon grant. This study is a product of the doctoral thesis in biotechnology "Producción biotecnológica de xilitol a partir de hidrolizados de raquis de palma con levaduras del género Candida sp."

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Relationship between color and physico-chemical properties of cashew apple (*Anacardium occidentale* L.) at different days of storage

Relación entre color y propiedades fisico-químicas del pseudofruto de caujil (*Anacardium occidentale* L.) en diferentes días de almacenamiento

https://doi.org/10.15446/rfnam.v74n2.90073

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Keywords:
Ascorbic acid
Cashew
Color analysis
Physico-chemical
Pseudo-fruit
Polyphenols

ABSTRACT

Cashew is a fruiting specimen exported from different countries, including Venezuela, due to its high nutritional value and unique taste. However, only the fruit (cashew nut) is considered the most used part for consumption while the pseudo-fruit (cashew apple) is discarded due to its strong taste and smell. This study is based on the changes that occur in the physical and chemical composition of the pseudo-fruit of cashew (*Anacardium occidentale* L.) in different days of storage (0, 3, 6, 9, 12) related to the color analysis of the pseudo-fruit. Image analysis was performed using the CIELab color space, which revealed different maturity stages for samples from day 0 to day 12. Nevertheless, antioxidant activity refers to ascorbic acid, and polyphenols content showed a degradation before day 6 of storage. These results prove that cashew apples can be stored for long-term at room temperature (25 °C), but the color and physico-chemical properties suffer some changes decreasing their nutritional value after day 6 of storage. A correlation between image analysis and chemical parameters can be used to evaluate the optimal maturity stage in samples.

Palabras clave:
Ácido ascórbico
Caujil
Análisis de color
Físico-quimico
Pseudofruto
Polifenoles

RESUMEN

El caujil o marañón es un espécimen fructífero exportado desde diferentes países, entre ellos Venezuela, debido a su alto valor nutricional y sabor único. Sin embargo, solo el fruto del caujil (nuez o semilla) se considera la parte más usada para consumo, mientras que el pseudo-fruto se desecha debido a su fuerte sabor y olor. Este estudio se basa en los cambios que se producen en la composición física y química del pseudo-fruto de caujil (*Anacardium occidentale* L.) en diferentes días de almacenamiento (0, 3, 6, 9, 12) relacionada con el análisis de color del pseudo-fruto. El análisis de imagen se realizó utilizando el espacio de color CIELab que reveló diferentes etapas de madurez para las muestras desde el día 0 al día 12. Sin embargo, la actividad antioxidante, que se refiere al ácido ascórbico y el contenido de polifenoles, mostró una degradación antes del día 6 de almacenamiento. Estos resultados prueban que los pseudo-frutos de caujil pueden almacenarse a largo plazo a temperatura ambiente (25 °C), pero el color y los cambios fisico-químicos provocan una disminución en el valor nutricional del pseudo-fruto después del día 6 de almacenamiento. Se puede utilizar una correlación entre el análisis de imágenes y los parámetros químicos para evaluar la etapa de madurez óptima en las muestras.

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Received: October 22, 2020; Accepted: April 01, 2021
Rev. Fac. Nac. Agron. Medellín 74(2): 9593-9602. 2021
ISSN 0304-2847 / e-ISSN 2248-7026

The cashew (*Anacardium occidentale L.*) is a product with a high content of flavors, colors, and the presence of fructose as the predominant sugar. It is cultivated in different parts of the world (Makanjuola *et al.,* 2013; Dedehou *et al.,* 2016) and in some Latin American countries, including Venezuela where it grows wild and without a protocol of cultivation (Guerrero and Velazquez-Moreira 2014; Guerrero *et al.,* 2019). These fruits represent an important part of the production of cashew nuts (fruit) but cashew apple is non-used in the food industry after the extraction of juice (Da Costa *et al.,* 2009). This characteristic is important in the commercialization of the pseudo-fruit due to the chemical characteristics of the cashew apple for a wide field of applications such as enriched powders (Da Costa *et al.,* 2009), nutritional juice (Das and Arora, 2017) and ethanol production (Oliveira *et al.,* 2019).

Recent studies in the chemical composition of cashew apple revealed an important amount of antioxidant among other compounds (Guerrero *et al.,* 2019; Oliveira *et al.,* 2019). Since the ripening index can only be calculated by employing a destructive technique using the calculation for soluble solids and titratable acidity (De Figueiredo *et al.,* 2002), image analysis emerges as a novel, easy, and low-cost technique that allows maturity calculations utilizing only a picture. This can be obtained using a single camera but the image analysis is the main part of the experiment, with several advantages over chromameters; such as a lower cost, the possibility of being used for in-line inspection, the capability of analyzing larger areas to provide spatial data, thus eliminating the subjectivity of human criteria (Orrillo *et al.,* 2019). Image analysis using a high-quality image and a region of interest (ROI) is a powerful tool for evaluating the quality of food by color (Tarlak *et al.,* 2016). It also allows developing a vast analysis of sample features such as size, shape, and image texture (Arzate-Vázquez *et al.,* 2011; Sa'ad *et al.,* 2015). It is well known that color is one of the most important appearance attributes in fruit and it has a high influence on consumer acceptability (Fernandes *et al.,* 2011). Some studies of cashew apples have not been established color as a quality criterion to determine the optimal maturity for harvesting and marketing (Vélez-Rivera *et al.,* 2014). These analyses have been related to the presence and concentration of pigments such as carotenoids, anthocyanins, flavonoids, and chlorophylls in other fruits (Corzo and Álvarez 2014).

The main objective of this contribution was to evaluate color and physico-chemical changes induced by different days of storage of cashew apples at room temperature through image analysis and established experiments, respectively. Results can prove a dependency between chemical parameters and color analysis, using only an image and a non-invasive experiment. These results can be reproduced in several pomaces, fruits, and other food in the market.

MATERIALS AND METHODS

Materials

Cashew (*Anacardium occidentale* L.) has two edible parts known as cashew nut (fruit) and cashew apple (pseudo-fruit), this part was used as a sample (Figure 1). 116 cashews were taken from a crop located on the outskirts of the Maracaibo city, Zulia, Venezuela. Samples were randomly and systematically taken from the crop in their respective ripening index according to Sindoni *et al.* (2009) and Gadani *et al.* (2017). Samples were transported and analyzed in the Centro de Investigaciones en Química de los Productos Naturales "Gladys León de Pinto", of Universidad del Zulia (L.U.Z.).

Fruit processing

Since cashews are perishable fruits, a physico-chemical analysis must be performed once the fruit is harvested, which means between 2 or 3 h after removed from the plant. To prevent any change in the physico-chemical evaluation, samples were selected and washed carefully with a sodium hypochlorite (NaOCl) solution (100 ppm) for 5 min (Gadani *et al.,* 2017). After that, a citric acid solution ($C_6H_8O_7$) (1% w/v) was used for 5 min to finish the cleaning in the samples, as Oliveira *et al.* (2020) reported. Samples were separated into three groups for the different treatments that would be applied and kept in storage around 23 °C for 0, 3, 6, 9, and 12 days of analysis. For physico-chemical analysis, pseudo-fruits were homogenized in a grinder machine (OSTER®, Model BRLY07-Z00, USA) and analyzed by triplicate in each day of storage. All analyses were performed on 0 day once the samples were ready to proceed. After that, samples were processed at 3, 6, 9, 12 days in the morning, and analyses were performed immediately.

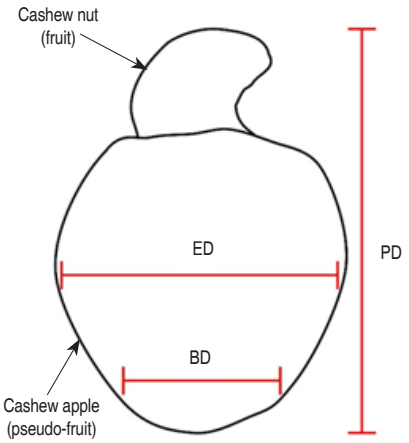

Figure 1. Representation of cashew nut (fruit) and cashew apple (pseudo-fruit). PD: polar diameter; ED: equatorial diameter; BD: basal diameter.

Color analysis

Images of the samples in each day of storage were used to evaluate color parameters. RGB images were captured with a size of 1800x2000 pixels and stored in the computer in TIFF format. ImageJ software version 1.51s (National Institutes of Health, Bethesda, MD, USA) was used to evaluate color changes in a selected region of interest (ROI) of each sample. CIELab color space was selected for color evaluation in the food industry due to its widespread use. It represents more closely the colors perceived by the human eye (Pathare *et al.,* 2013). Thus, CIELab coordinates, L* (luminosity), a* (green to red), and b* (blue to yellow) were estimated. Previously, the digital camera was calibrated using the values of CIELab coordinates (L*, a*, b*) evaluated by image analysis and a chromameter (CR400, Minolta, Japan) (images of 35 color patches) (Arzate-Vázquez *et al.,* 2011). For each color parameter, the values of 35 color patches obtained by image analysis and chromameter were correlated with a linear fit. For the three plots of color coordinates (L*, a*, b*), R^2>0.95 were obtained.

For the calibration procedure and image analysis of cashews, the conversions were performed using the "Color Space Converter" plugin of ImageJ 1.51s to convert RGB images into L*, a*, b* separate channels. Illuminant D65 was used in the image analysis as well as in the evaluations with the chromameter. The values from each CIELab coordinate were obtained using the histogram tool of ImageJ software.

Additionally, chroma values (C*) and Hue angle (H*) were calculated with Equations 1 and 2 using values for CIELab color space.

$$C^* = \sqrt{(a^*)^2+(b^*)^2} \quad (1)$$

$$H^* = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (2)$$

Also, color differences (ΔE) were calculated from equation 3 (Arzate-Vázquez *et al.,* 2011):

$$\Delta E = \left[(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2\right]^{(1/2)} \quad (3)$$

where $\Delta L^* = L^* - L_0^*$; $\Delta a^* = a^* - a_0^*$; $\Delta b^* = b^* - b_0^*$, being L_0^*, a_0^*, b_0^* the color parameter values of the samples at day 0 and L*, a*, b* the color parameter values of the fruits at different days of storage.

Physico-chemical analysis

Mass content. Samples were weighed in an electronic balance (Mettler Pc. 4400, USA) on 0, 3, 6, 9, and 12 days. Mass content (MC) was calculated for each sample group in an average calculation and the results were expressed in g $fruit^{-1}$ (Guerrero *et al.,* 2008).

Diameter calculations. Samples for each group were measured using a digital Vernier (Fisher Scientific, USA) in three different points of the fruits (Figure 1). Polar diameter (PD), equatorial diameter (ED), and apical diameter (AD) were measured on the first day (0 days)

and the different days of storage (3, 6, 9, 12 days). Values were reported on average for three samples and results were expressed in mm (Guerrero *et al.,* 2008).

Chemical analysis. The total soluble solids (TSS), titratable acidity (TA), moisture content percent M (%), and pH of the cashew apples were evaluated in the fresh pulp (pseudo-fruit). Homogenized samples were prepared for each day of storage and pH was evaluated in each sample using the 1315-79 standard COVENIN (1979). TSS was measured using a digital refractometer (Carls Zeiss, 130486, USA) using the AOAC (2019) with the procedures 22.024/31.011. TA was determined by titration with 1 N NaOH, the values were expressed as malic acid g 100 g^{-1} of the sample as 1151-77 standard COVENIN (1977) reported. In samples of 0, 3, 6, 9, and 12 days, M (%) was measured using the weight of the samples before and after the drying process at 80 °C in triplicate using AOAC (2019) in the procedures 22.008, 22.013.

Ascorbic acid and phenolic content determination. The ascorbic acid content (AAC) was measured using the Tilsman method AOAC (2019) (43.060) and reported in mg of ascorbic acid 100 g^{-1} of the sample. Phenolic compounds (PC) were extracted using methanol as Hernández-Varela *et al.* (2013) previously reported. The supernatant was kept in storage in an amber flask at -15 °C and analyzed 48 h before the extraction. A calibration curve from 0 to 500 µg L^{-1} was made using gallic acid as standard and the results were expressed in mg L^{-1} of gallic acid equivalents (GAE).

Statistical analysis

An analysis of variance (ANOVA) with all multiple pairwise comparisons with Tukey's test in SigmaPlot software version 11.0 (SYSTAT Inc., USA) was performed. No significant differences were considered when $P<0.05$.

RESULTS AND DISCUSSION

Color analysis

Color analysis in food samples is a quality control parameter that must be evaluated to know the visual appearance and organoleptic acceptance of the samples (Medina *et al.,* 2010; Vélez-Rivera *et al.,* 2014). Figure 2 shows a color gallery of cashew fruits at different days of storage (0, 3, 6, 9, 12 days). A stable comparison of maturity for the samples was observed. Lopes *et al.* (2012) explained that pigments present in the samples (carotenoids and anthocyanins) found during the ripening reflect the changes in the color of the cashew apple.

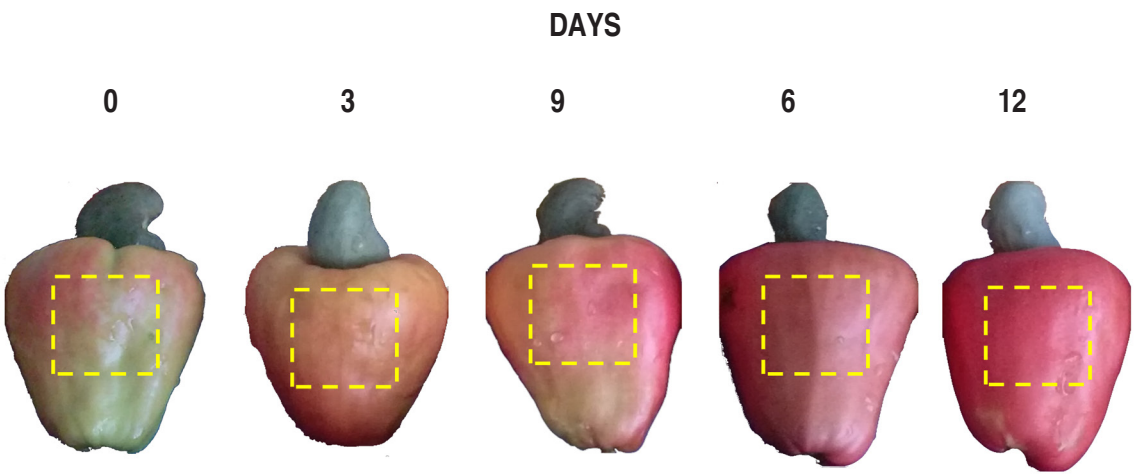

Figure 2. Color images of cashew fruits and pseudo-fruits at different days of storage. ROI: region of interest in a yellow dashed square.

Image analysis was performed using the above-mentioned Figure 2. Changes in the color space coordinates (L*, a*, b*) for each day of storage and their differential of color (ΔE), Chroma values (*C) and Hue angle (H*) were evaluated. Figure 3 shows the L*, a*, b* values obtained with the CIELab color space

plug in for the ROI pictured in the image, and later on, the ΔE values, hue angle, and chroma values of the images for each day of storage were calculated. Figure 3a-c shows values of L*, a*, b* parameters in cashew apples, these data revealed the same tendency from a lower to a higher maturity as other authors have referred (Lopes *et al.,* 2012). Changes in the value of L* (Figure 3a) are related to the brightening of the samples (Hernández-Hernández *et al.,* 2016) and it can be appreciated that L* values in all the samples remain similar, although changes in the a* and b* values are observed. Values of b* fluctuates from day 0 to day 12, starting with an increase until day 6 and later on a reduction in day 9, which concludes with an increase in day 12. These values are related to changes from blue tones (-b*) to yellow tones (+b*) in the samples. On the other hand, a* values are related with yellowish tones (-a*) to reddish tones (+a*) (Pathare *et al.,* 2013). It can be observed that in maturity, the most important compounds responsible for color changes are carotenoids and anthocyanins, being carotenoids the responsible for changes in yellow-red tones, and anthocyanins responsible for changes in blue-yellow and blue-red tones, depending on pH (Khoo *et al.,* 2017; Ranganath *et al.,* 2018). Figure 3b corroborates an increase of red tones from 0 to 12 days, reflecting a major presence of carotenoids in the sample due to the ripening of the samples. Besides, Figure 3c shows a slight increase of yellow tones from 0 to 12 days, related to a major presence of anthocyanins. Khoo *et al.* (2017) explained that in acidic conditions, anthocyanin appears as red pigment while blue pigment anthocyanin exists in alkaline conditions. Lopes *et al.* (2012) described that carotenoid content in their samples increased during ripening without any significant difference. The color changes in this study are similar to those reported before.

Figure 3D shows a comparative result of the differential of color (ΔE) for the initial time of storage (day 0) concerning the color of the samples at a different time of storage (3, 6, 9 12, days). For 6 and 9 days, a clear stationary result is presented due to the reach of a

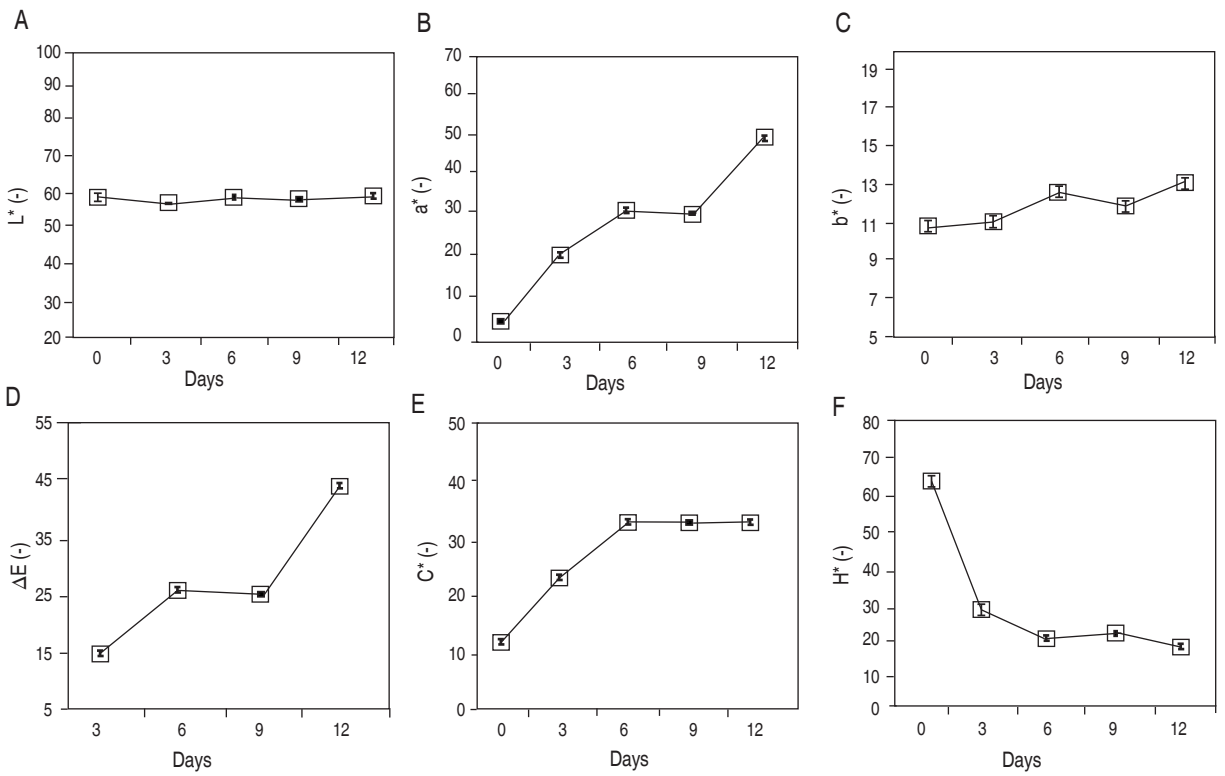

Figure 3. Image analysis of cashew apple. L* (A), a* (B), and b* (C) coordinates of CIELab color space; (D) ΔE values, (E) C*: chroma values, (F) H*: hue angle values in degrees.

proper maturity of the samples. ΔE<15 is associated with yellowish color in the samples and a more reddish tone is found when ΔE>30 (Oliveira *et al.,* 2019). Results of ΔE=26 in this study demonstrated an accurate stage of maturity in the samples.

A remarkable opinion-based on image analysis of fruit samples could be discussed since color changes are used as a quality parameter, this non-destructive technique provides a huge amount of information of the sample and could be extended to other fruits with nutritional and commercial importance.

Chroma value (C*) increased from day 0 to day 6, and after that time it remained stable until the last day of storage (Figure 3e). As usual, the color became more intense due to the reactions of the ripeness that generate representative color changes but after a specific time, those changes are less observable (Fernandes *et al.,* 2011). Besides, the hue angle (H*) traditionally defines colors as rosy, yellowish, and greenish (Pathare *et al.,* 2013). The cashew apples had a percentage reduction of 76% from day 0 to the last day of storage (day 12) (Figure 3F). Day 0 is more related to a yellow pseudo-fruit (90°) and at the end of maturity, it is more related to a red value (0°) (Oliveira *et al.,* 2019). Comparing H* with C* values, it is possible to infer that after day 6, more stable changes are shown and these values can be used as a good parameter to select a sample ready for consumption following the results obtained in ΔE and a* parameters.

Physical analysis

Figure 4 shows the average results obtained from the physical analysis of the pseudo-fruits. Taking into account that between days 6 and 9 is the best stage of maturity for the samples, these values are discussed below. The average of ED in the samples was 54.20 mm on day 0, followed by a reduction on day 3 and an increase on day 6. This behavior is typical in samples with a higher amount of water in the cells (Meyers *et al.,* 2008). Expansions of the samples are common when samples are at a controlled temperature, and other reactions are present inside the fruit (Adjou *et al.,* 2017). The values of PD are more heterogeneous due to the proper size of the samples, but specimens showed that after day 6,

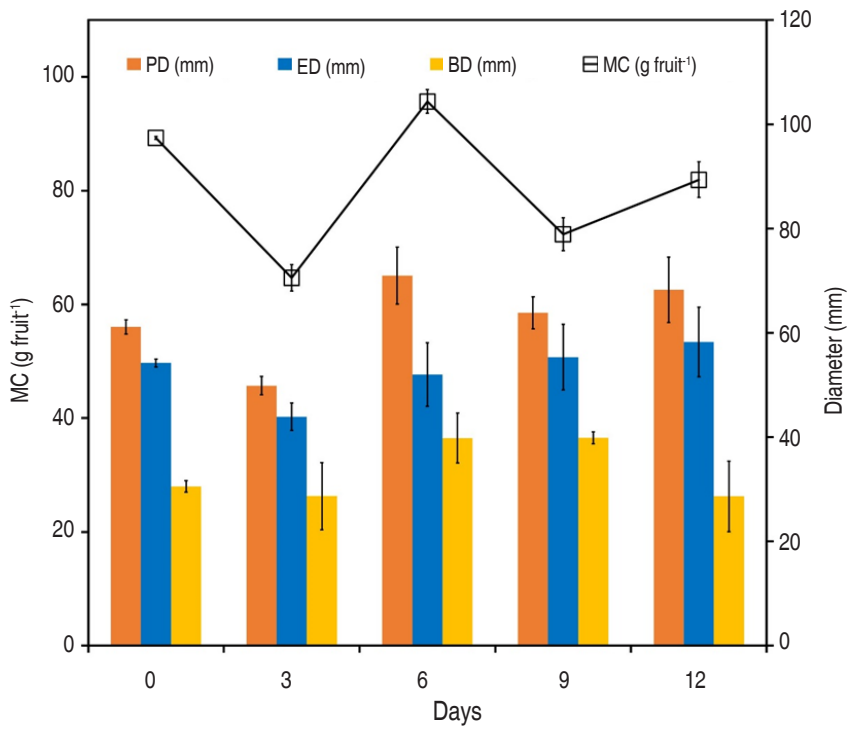

Figure 4. Physical analysis on cashew apple. MC=mass content; PD= polar diameter; ED= equatorial diameter; BD= basal diameter.

compression due to dehydration of the sample is found (Fernandes *et al.,* 2011). Changes in the BD follow a similar tendency between day 0 and day 6; and after that day, samples tend to be compressed and become smaller. Finally, the mass content (MC) from day 0 (89.25 g fruit^{-1}) had a similar tendency until day 6 in which a higher value is found (95.67 g fruit^{-1}). Later, day 12 revealed that MC decreases until 81.90 g fruit^{-1} and these values are explained as a common reaction on the size changes of the fruit when a maturity process is occurring (Singhal *et al.,* 1997). All values showed significant differences (P<0.05) between each day of storage. A comparison with matured *Anacardium occidentale* from India proposed by Das and Arora (2017) reported that mass (50-140 g fruit^{-1}), width (35-50 mm), length (40-75 mm) are similar to those values obtained in this study.

Chemical analysis

Table 1 shows the pH, TSS, TA, M, AAC, and PC of pseudo-fruits of *A. occidentale* used in this study. The pH of cashew apples ranged from 3.74 to 4.10. These values are reported to be considered in the acidic range for food (Oliveira *et al.,* 2019). Due to particularities in the pH of the samples discussed above, it is possible to find a redder tone in the last stage of maturity when samples tend to be more acidic. These results proved there is a higher concentration of anthocyanins at the end of the ripening taking into account the color of the pseudo-fruits (Khoo *et al.,* 2017). TSS in cashew apple were in a higher range of 10.69 to 18.82 ºBrix, comparable with those obtained in *A. Othonianum* accessions between 9.60 - 13.47 ºBrix (Oliveira *et al.,* 2019). TA for the samples was established in a range of 0.21 to 0.37 mg 100 g^{-1} of malic acid. Makanjuola *et al.* (2013) reported a range of 0.22 to 0.29% after 28 days of preservation in cashew apple juice at room temperature.

Moisture content M(%) follows the typical decrease tendency for fruits in which a loss of water is observed during the storage time (Hosseinpour *et al.,* 2011). For AAC, a high level of vitamin C was observed in the first 6 days of storage since ascorbic acid remains more stable in an acidic medium (Makanjuola *et al.,* 2013) but after this time other reactions inside of cashew juices can produce a loss of vitamin C in the storage time (Oliveira *et al.,* 2019). The same behavior is observed for PC, where higher values are observable in the first days of storage (0-6 days) but after that time, a small decrease in the content was observed similar to Lopes *et al.* (2012) report for cashew apples in a ripening value of 7.

Table 1. Chemical parameters for cashew apple (*Anacardium occidentale* L.) at different days of storage.

Chemical analysis	Days				
	0	3	6	9	12
pH	3.84± 0.05 a	4.10± 0.02	3.77± 0.03 a	3.77±0.01 a	3.74± 0.03 a
TSS	10.69± 2.42	13.52± 1.51	18.82± 1.13 b	18.14±0.82 b	16.97± 1.02
TA	0.21± 0.05	0.26± 0.03	0.37± 0.02 c	0.36±0.01 c	0.35± 0.02 c
M	87.75± 0.54 d	87.12± 1.16 d	84.85± 2.36	76.82±4.28	73.73± 0.30
AAC	426.26± 10.57	467.94± 12.50	444.34± 7.25	286.55±2.41	272.35± 5.05
PC	463.05± 6.48	474.00± 12.81	439.48± 6.04 e	422.57±3.11	436.53± 0.36 e

*Values with the same letter in a row have not significant differences (P<0.05).

TSS=total soluble solids reported in °Brix; TA=total acidity expressed in g of malic acid 100 g^{-1} sample; M=moisture content reported in %; AAC=ascorbic acid content expressed in mg of ascorbic acid 100 g^{-1} sample; PC=polyphenol content expressed in mg L^{-1} of gallic acid equivalents (GAE)

Color and chemical correlation

On the other hand, fruits with low acid and high soluble solid contents are desirable for raw consumption (Malevski *et al.,* 1977). However, the pseudo-fruits are richer in the antioxidant compounds only at the initial stage of maturity (Table 1, AAC, and PC values). The authors reported that the anthocyanins, total carotenoids, TSS, TSS/TA, polymeric tannins, vitamin C, and reducing sugars increase

continuously during the maturation of the cashew apples (Makanjuola *et al.,* 2013; Dedehou *et al.,* 2016; Das and Arora 2017). These results are proof of the behavior of the samples in this study, since values increased from day 0 to day 6 and later on, decreased on days 9 to 12. With this explanation is easy to achieve a well-matured and perfect consumable fruit before day 6 of storage, based on the physical and chemical results previously presented.

The color values and the AAC and PC values in Table 1, can be directly related to Lopes *et al.* (2012) results, carotenoid pigments display a considerable level of antioxidant activity as well as the total anthocyanins content that also increases with ripening. Finally, physico-chemical characteristics of cashew apple in pulp and juice form, have differences based on the locations of harvest. These differences are associated with changes in soil conditions, cultural practices, and other climatic conditions such as temperature and humidity (Makanjuola *et al.,* 2013; Oliveira *et al.,* 2019).

Figure 5 shows the correlation between the total antioxidant activity (AAC and PC values) with the image analysis (hue angle values) that are related to the changes in the tonality of the samples at different days of storage. The correlation reveals an interesting behavior for AAC vs H* (Figure 5a) and PC vs H* (Figure 5b). The changes in color for cashew pseudo-fruits was explained as a decrease of yellowish tones in the early stages of ripening until an increase in red tonalities was observed due to the intensification of anthocyanins, anthocyanidins, and other compounds that appears as part of the maturity (Khoo *et al.,* 2017). But, authors report that antioxidant activity (AAC and PC) should gradually increase with the rise of polyphenols and other antioxidant compounds that are present in the sample (Queiroz *et al.,* 2011; Makanjuola *et al.,* 2013). For ACC, the rise of these levels from day 0 to day 3 is attributed to an ascorbate oxidase reduction, which is an enzyme responsible for its degradation, or to a decrease in the levels of Cu^{2+}, which is a cofactor for the enzyme (Lopes *et al.,* 2012). Nevertheless, AAC and PC revealed that after day 6 of maturity, the values started to decrease and showed a stable value for AAC at the end of the maturity stage (Figure 5a) but for PC a fluctuation was observed at the end of the maturity stage (Figure 5b). Since, the hue angle (H*) in the images analysis confirms the appearance of reddish tones in the sample, it could be explained that antioxidant capacity is reduced due to the internal reaction of the pseudo-fruits and inner senescence of the tissues. According to Scheer (2013) when the chlorophyll is lost and other pigments are synthesized, color changes during maturation are produced.

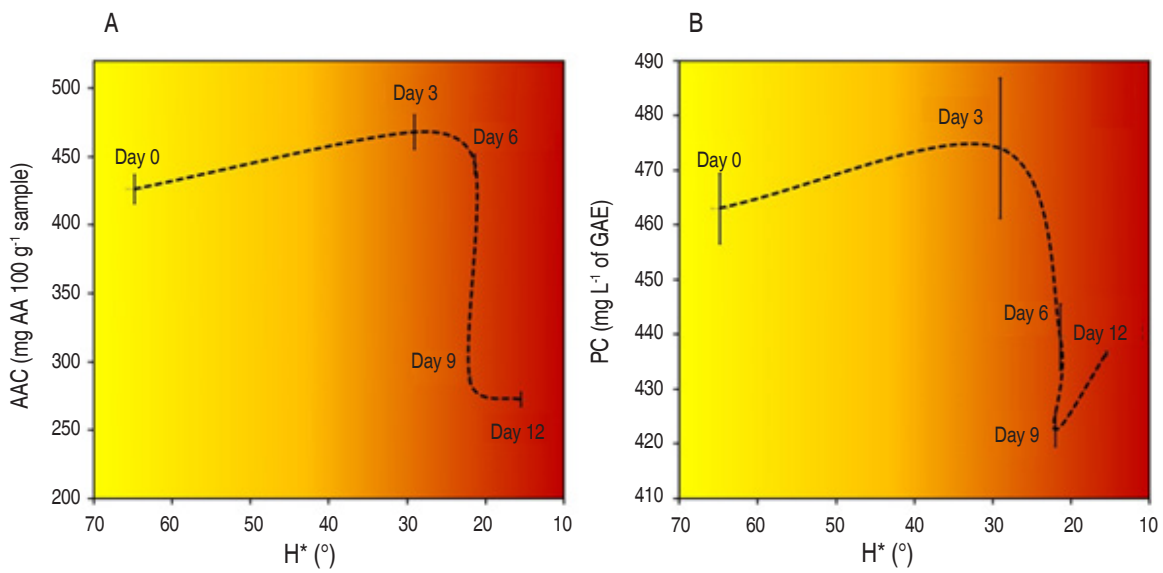

Figure 5. Correlation between hue angle (H*) and (a) the ascorbic acid content (AAC) and the polyphenolic content (PC) in the pseudo-fruits on different days of storage. Yellow tones are on day 0 and red tones on day 12.

CONCLUSION

Since cashews are a perishable fruit with a lower facility of consumption than other fruits, an important market could be developed when the physical and chemical characteristics of the samples are evaluated. Image analysis reveals that cashew apples are good material to develop the analysis with high accuracy, also this technique emerges as an easy and non-invasive technique to select a sample with a consumption maturity without resorting to a destructive technique. Then, using only an image is possible to correlate the hue angle values (<30°) associated with the tonality of color changes in the fruit and follow the antioxidant capacity of the sample. Finally, this fruit represents a new alternative to extract more stable and rich antioxidant compounds after 6 days of storage (444.3±7.25 mg AA 100 g^{-1} sample AAC and 439.48±6.04 mg L^{-1} of GAE PC), that could be employed in the development of new food products with an enrichment in the crop cost, due to the lower consumable and practicality of this sample.

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Revista Facultad Nacional de Agronomía

Evaluation of resistance to *Fusarium oxysporum* in genotypes of lulo (*Solanum quitoense* Lam.)

UNIVERSIDAD NACIONAL DE COLOMBIA

Evaluación de resistencia a *Fusarium oxysporum* en genotipos de lulo (*Solanum quitoense* Lam.)

https://doi.org/10.15446/rfnam.v74n2.90447

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Keywords:
Inoculation
Resistant genotypes
Solanum hirtum
Tolerance
Vascular wilt

ABSTRACT

Lulo (*Solanum quitoense* Lam.) is a fruit tree of Andean origin of national economic importance in Colombia, which constitutes an important source of employment for farmers and their families. Vascular and root wilt caused by the fungus *Fusarium oxysporum* is one of the most limiting diseases in the production of this species, causing low yields and considerable economic losses. As an effective control alternative for this pathogen, the identification of genotypes with resistance that can be used in breeding programs is being considered. The objective of this work was to evaluate the response of 22 lulo genotypes to the artificial inoculation of *Fusarium oxysporum* to identify possible sources of pathogen tolerance. *F. oxysporum* was inoculated on 22 genotypes of lulo plants following the method of wounded roots through artificial cutting. Distilled water inoculation and "La Selva" resistant lulo hybrid was used as control. The traits evaluated correspond to plant height, stem diameter, days to the onset of symptoms, incidence and severity. The fungus isolation was highly aggressive in *S. quitoense* and *S. hirtum*, with 96% and 84% severities, respectively. Five resistant genotypes were identified, namely 15C, 36B, HSF1, HSF10, and HSF36, which presented incidences and severities of 0% and can be considered for multi-environmental evaluation tests to determine their productive potential or they can be considered as parents for breeding programs of the species. Other genotypes, such as 15B and HSF15, showed average severities of 28% and 20%, respectively; however, these two genotypes survived throughout the experiment, suggesting tolerance to the pathogen.

Palabras clave:
Inoculación
Genotipos resistentes
Solanum hirtum
Tolerancia
Marchitamiento vascular

RESUMEN

El lulo (*Solanum quitoense* Lam.) es un frutal de origen Andino de importancia económica en Colombia, que constituye una fuente importante de empleo para los agricultores y sus familias. El marchitamiento vascular y radicular causado por el hongo *Fusarium oxysporum*, es una de las enfermedades más limitantes en la producción de esta especie, ocasionando bajos rendimientos y pérdidas económicas considerables. Como alternativa de control efectiva para este patógeno, se considera la identificación de genotipos con resistencia que puedan ser usados en programas de mejoramiento. El objetivo de este trabajo fue evaluar la respuesta de 22 genotipos de lulo a la inoculación artificial de *Fusarium oxysporum* para identificar posibles fuentes de tolerancia al patógeno. *F. oxysporum* fue inoculado en 22 genotipos de lulo siguiendo el método a la raíz con cortes artificiales. Inoculación con agua destilada y el hibrido "La Selva" fueron usados como control. Las variables evaluadas corresponden a la altura de planta, diámetro del tallo, días al inicio de síntomas, incidencia y severidad. El aislamiento del hongo fue altamente agresivo sobre *S. quitoense* y *S. hirtum*, con una severidad del 96% y el 84%, respectivamente. Se identificaron cinco genotipos resistentes, 15C, 36B, HSF1, HSF10 y HSF36, que presentaron incidencia y severidad del 0% y pueden ser considerados para evaluaciones multi-ambientales para determinar su potencial productivo o como parentales en programas de mejoramiento de la especie. Otros genotipos, como el 15B y el HSF15, mostraron una severidad media del 28% y el 20%, respectivamente; sin embargo, estos dos genotipos sobrevivieron durante el experimento, lo que sugiere una tolerancia al patógeno.

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Received: January 26, 2021; Accepted: March 08, 2021
Rev. Fac. Nac. Agron. Medellín 74(2): 9603-9614. 2021

ISSN 0304-2847 / e-ISSN 2248-7026

Lulo (*Solanum quitoense* Lam.) is a crop that belongs to the section *Lasiocarpa*, family Solanaceae, and genus *Solanum.* Its center of origin is located between the Colombian and Ecuadorian Andes (Heiser and Anderson, 1999; Lobo and Medina, 2000; Ramírez and Davenport, 2020; Ramírez 2021). Several authors, including to Ramírez *et al.* (2018); Arias and Rendón (2014); Muñoz *et al*. (2013); Paull and Duarte (2012) and Medina *et al*. (2009), mention various factors that make lulo a promising fruit-tree with high productive potential, including a high genetic variability with related species in the Andean region, proper niches for planting, consumer acceptance of the fruit and agroindustrial potential.

Despite the productive potential of lulo, there is a lack of development of this crop. According to Agronet (2019), Colombia has a planted area of 8,821.35 ha with a production of 89,050.41 t and an average yield of 10.1 t ha^{-1}. The main producers are the departments of Huila, Valle del Cauca, Antioquia and Boyacá, with production ranging from 8,424 to 14,339.8 t. The cultivated area, production and yield in the department of Nariño have decreased considerably from 2007 to 2016 about of 6, 17 and 12%, respectively. This behavior may be caused, among others, by the dynamics of production systems based mainly on a scheme lacking advanced technologies and a shortage of planting materials (Almanza *et al.,* 2016), since genetic improvement programs in the region have been directed primarily to industrial crops and crops of importance in food security (Pareja *et al.,* 2010). For this reason, despite having suitable soil and climatic conditions for the crop, it does not reach its potential under competitive growing conditions (Casierra *et al.,* 2013).

One of the most important factors that has reduced the productivity of lulo crops in the department of Nariño, especially in cultivars (e.g. Castilla and Larga Vida) planted by growers, is the susceptibility to vascular and root wilt caused by *Fusarium oxysporum*. In most cases, this problem reduces productivity, delays the crop cycle, and leads the death of many plants. Therefore, genotypes with resistance genes that allow counteracting the disease must be identified and selected (Díaz *et al.* 2011). *F. oxysporum* has caused economic losses ranging from 50 to 90% in commercial crops (Lagos *et al*. 2015), and it is characterized by visible symptoms, such as chlorosis and loss of turgor, causing complete wilting of the plant that develops from the germination of chlamydospores that come into contact with the roots, generate an appressoria that penetrates the root cortex and develops an internal mycelium in the root and advances until it reaches the vascular system (xylem ducts) (Cruz *et al*. 2011). Internally, the vascular bundles display discoloration or ascending necrosis that is evident through a cross-section of the main stem (Ochoa, 2002). This disease is considered difficult to manage since *F. oxysporum* can survive in plant residues and soil due to resistant structures known as chlamydospores (Agrios, 2005). The survival period of this fungus can be up to 20 years, triggering the loss of soil productivity for new plantings (Estupiñan and Ossa, 2007).

The control of this pathogen generally involves the excessive use of chemical fertilizers that affect human health and cause further environmental degradation (Villa-Martínez *et al.* 2014). Therefore, in an integrated crop management approach, disease management, environmental protection and reduction of the use of agrochemicals are prioritized actions, requiring strategies that include agronomic and cultivar improvement practices that take advantage of the diversity associated with the species. However, these practices require the adoption of processes and technology transfer, which are often difficult to implement (Ochoa, 2002; Ochoa and Gallardo, 2004; Clavijo, 2014; Arizala *et al*., 2011; Cardona, 2013).

A viable option for disease management is genetic resistance, in which the assessment of genotypes focuses on the search for sources of resistance. This alternative provides many advantages, for instance, it is environmentally friendly, reduces the possibility of developing pathogen resistance to agrochemicals, and provides low production costs due to the reduced use of agrochemicals (Clavijo, 2014).

Several studies on different commercial species have studied the sources of resistance to *F. oxysporum* obtaining a great contribution to the genetic improvement processes, such as García-Velasco *et al.* (2020), who evaluated the resistance or susceptibility of *Musa* sp. cultivars in Cuba, using different filtrates of *F. oxysporum*, allowing the establishment of an efficient

and non-destructive method for the identification of races 1 and 2 of this pathogen, Carvalho *et al*. (2021) identified *Passiflora nitida, P. foetida* and *P. mucronatacan* species as sources of resistance to *F. oxysporum* and *F. solani* complex and recommended them for use in *Passiflora* breeding programs of Brazil and Shaw *et al.* (2017) identified a dominant nature of resistance in *Ricinus communis* inbred line AP42 to *F. oxysporum* f. sp. *ricini*, which is of great interest in hybridization programs in India, among others.

At national level, studies carried out by Tamayo *et al.* (2002) assessed the genetic resistance of the interspecific lulo hybrid "La Selva" (*S. quitoense* x *S. hirtum*); in particular, the authors recommend this material as a control alternative, with economic impact, in areas where the development of this crop is hindered by the pathogen. In other Solanaceae, such as *Capsicum* spp., Clavijo (2014) identified six accessions with resistance genes, which are recommended as parental plants in chili pepper improvement programs. Morales *et al*. (2014) assessed wild and cultivated accessions of *Solanum lycopersicum* and found sources of resistance in *Solanum neorickii*; hence, the authors recommended this species as a parent in interspecific breeding programs. Mayorga-Cubillos *et al.* (2019) identified six genotypes of cape gooseberry (*Physalis peruviana*) that are promising against *F. oxysporum* and can be used in subsequent breeding schemes.

In this context, the study hypothesis is based on the fact that within the working collection of lulo there are no genotypes with characteristics of tolerance or resistance to the artificial inoculation of *F. oxysporum* and the objective of this work was to evaluate the response of 22 lulo genotypes, from the GPFA (Grupo de Investigación en Produccion de Frutales Andinos) collection, to the artificial inoculation of *Fusarium oxysporum* Schelcht, the causal agent vascular and root wilt and to select sources of resistance that can be useful in lulo breeding programs.

MATERIALS AND METHODS

Location

The experiment was carried out at the greenhouse of Botana Experimental Farm of Universidad de Nariño at 2,670 masl (01°09'30.62"NL, 77°16'31.79"WL), with an average outdoor temperature of 12°C, average indoor greenhouse temperature of 22°C and 80% relative humidity.

Plant material

As plant material, 22 lulo genotypes were used, including 10 selected based on field-resistance to *F. oxysporum* and good agronomic traits obtained by sexual seed propagation (Table 1), as well as 12 genotypes from half-sibling families (HSF) propagated by cuttings were evaluated. The 22 genotypes belonged to the GPFA collection of Universidad de Nariño (Table 2).

Table 1. Field-resistance lulo genotypes to *Fusarium oxysporum* derived from sexual seed, under natural conditions of the region of the department of Nariño, Colombia.

Code	Genotype	Pedigree
15B	UDENAR-SQEFma015	UDENAR-SQEFma015 La Florida/i3-LF014
15C	UDENAR-SQEFma015	UDENAR-SQEFma015 La Florida/i8-LF014
16A	UDENAR-SQEFma016	UDENAR-SQEFma016 La Florida/i2-LF014
19A	UDENAR-SQEFma019	UDENAR-SQEFma019 La Florida/i5-LF014
22A	UDENAR-SQEFma022	UDENAR-SQEFma022 La Florida/i1-LF014
35A	UDENAR-SQm035	UDENAR-SQm035 La Florida/i3-LF014
37A	UDENAR-SQm037	UDENAR-SQm037 La Florida/i3-LF014
37B	UDENAR-SQm037	UDENAR-SQm037 La Florida/i4-LF014
38C	UDENAR-SQm038	UDENAR-SQm038 La Florida/i8-LF014
42A	UDENAR-SQm042	UDENAR-SQm042 La Florida/i3-LF014

The 12 HSF (Table 2) are derived from two selection stages. In the first stage, a commercial crop plantation was established, using seeds of the "La Selva" cultivar, located in Nariño (01°07'24.28"N,77°26'19.66"W), at 2,237 masl. A stratified selection was made from this plantation; the best plant was selected from each stratum according to yield, fruit quality, health and architecture. To create 50 HSF, 50 plants were selected.

Table 2. Half-sibling families of lulo derived from cuttings, obtained through individual stratified selection of the region of the department of Nariño, Colombia.

HSF	Pedigree	Location in selection strata
1	Y1113	Yacuanquer, strata 1, substrata 1, furrow 1, plant 3
2	Y1242	Yacuanquer, strata 1, substrata 2, furrow 4, plant 2
4	Y1442	Yacuanquer, strata 1, substrata 4, furrow 4, plant 2
7	Y2221	Yacuanquer, strata 2, substrata 2, furrow 2, plant 1
10	Y2432	Yacuanquer, strata 2, substrata 4, furrow 3, plant 2
15	Y5231	Yacuanquer, strata 5, substrata 2, furrow 3, plant 1
22	Y8111	Yacuanquer, strata 8, substrata 1, furrow 1, plant 1
25	Y8523	Yacuanquer, strata 8, substrata 5, furrow 2, plant 3
28	Y10122	Yacuanquer, strata 10, substrata 1, furrow 2, plant 2
29	Y10232	Yacuanquer, strata 10, substrata 2, furrow 3, plant 2
36	Y(1)5.17	Yacuanquer, lote 1, furrow 5, plant 17
45	Y(2)2.2	Yacuanquer, lote 2, furrow 2, plant 2

In the second stage, 50 HSF were planted in experimental trials in four localities of the department of Nariño, namely La Unión, San Pedro de Cartago, Arboleda and Tangua, which are located between 1,700 and 2,100 masl. The experiment was a completely randomized block design with three repetitions and the families as treatments. At each locality, growth, yield, and fruit quality were assessed as the response variables. The data were analyzed through an Analysis of Variance and a selection index was applied to the most important variables. Based on this, 12 HSF were selected and propagated taking 15 cm long, healthy cuttings with at least two axillary buds that were planted in sterile substrate for rooting and then, evaluated by their response to artificial inoculation by *F. oxysporum.*

Inoculation of the lulo genotypes

Selected lulo genotypes were inoculated with *F. oxysporum* (Fo) isolate Fo15, collected in the municipality of Buesaco, Nariño, Colombia and evaluated for its pathogenicity on *S. quitoense* and *S. hirtum*, generating severity of 96% and 84%, respectively (Duarte-Alvarado *et al.,* 2020). The five inoculated plants per genotype showed at least four true leaves and a root growth without phytosanitary problems. The inoculation method based on wounded roots was applied (Clavijo, 2014), which consisted in washing the roots with distilled water, making small cuts in the apices, and submerging the roots in previously prepared inoculum for 3 h, then, the plants were sowed in a sterile substrate. The control was subjected under the same treatment, except the immersion step in sterile distilled water (Betancourth, 2005; Clavijo, 2014).

For the preparation of the inoculum, a spore suspension of pure monospore cultures grown in PDA at 28°C for 7 to 10 days was used. Subsequently, 20 mL of sterile distilled water was added and a superficial scraping with a sterile spatula to remove conidia and mycelium. Afterwards, the solution was passed through a filter to separate the conidia from the mycelium and adjusted to a concentration of $1x10^6$ conidia mL^{-1} by a haematocytometer with a Neubauer chamber (Clavijo, 2014).

The plants with symptoms of vascular and root wilt caused by *F. oxysporum* were taken to the laboratory to confirm

Koch's postulates (Koch, 1876). The genotypes that did not show disease symptoms and survived during the trial period were considered to be resistant. Also, to confirm these results, re-inoculations with a mix of four isolates, namely Fo1, Fo2, Fo16, and Fo19, which cause 100% severity in *S. quitoense* were performed according to the study carried out by Duarte-Alvarado *et al.* (2020).

Experimental design

A completely randomized design with five repetitions with the 22 lulo genotypes (Tables 1 and 2) as treatments was implemented. Each repetition comprised five plants. The experimental unit corresponds to a plant grown in a pot with sterile substrate (pH of 5.5, 88.7% organic matter, 758.29 ppm of N, 499.35 ppm of P, 2,371.25 ppm of K), 110 experimental units in total. Two controls were used; the inoculation control was Castilla cultivar inoculated with distilled water and Fo15 and the resistant control was "La Selva" hybrid inoculated with isolate Fo15 and sterile distilled water (Tamayo *et al.,* 2002).

Disease assessment

The response of the genotypes to the inoculation by measuring the following variables:

- Plant height (PH) (cm) was measured for the genotypes propagated by seeds (Table 1). Following inoculation, the PH was recorded every seven days for three months. After the increase in PH, the ΔPH was calculated through the Equation 1:

$$\Delta PH = \frac{PH2 - PH1}{T2 - T1} \quad (1)$$

Where: PH1=plant height in cm at T1, AP2=plant height (cm) at T2, T1=initial time of PH measurement, T2=final time of PH measurement.

- Stem diameter (SD), similar to PH, SD (cm) was assessed for the genotypes propagated by seeds (Table 1). Following inoculation, the SD was recorded every seven days for three months. The increase in SD (ΔSD) was calculated through the Equation 2:

$$\Delta SD = \frac{SD2 - SD1}{T2 - T1} \quad (2)$$

Where: SD1=stem diameter in cm at T1, SD2=stem diameter in cm at T2, T1=initial time of SD measurement, T2=final time of SD measurement.

Considering the nature of the reproduction of the genotypes evaluated, the variables PH, SD were taken only in those genotypes multiplied by sexual seed (Table 1), given that the root system of plants propagated by cuttings does not allow these two variables to be taken with precision.

The following variables were evaluated for the 22 genotypes, including 10 derived from sexual reproduction (Table 1) and 12 from asexual reproduction (Table 2):

- Days to the onset of symptoms (DOS), number of days from the moment of inoculation to the onset of disease symptoms.

- Incidence (I) expressed as percentage, based on the number of diseased plants divided by the total number of plants assessed.

- Severity (S) developed by each plant during 84 days of evaluation was calculated based on the scale proposed by Elmer and Robert (2004) (Table 3).

Table 3. Scale for weighted severity assessment of *Fusarium oxysporum*.

Scale	Symptoms
0	No visible symptoms of the disease (Healthy plant)
1	Mild leaf chlorosis
2	<10% of plants with mild chlorosis and/or mild growth retardation
3	11-25% of plants with mild chlorosis and/or mild stunting and wilting
4	26-50% with strong chlorosis and/or dwarfism and withering
5	51-100% of the plants withered or died

The average S(%) was calculated according to the Equation 3 (Song *et al*., 2004), which is estimated according to the scale in Table 3 and expresses the severity as an index.

$$S(\%)=\left[\frac{\sum(\text{Number of diseased plants x each degree of illness}}{\text{Number of plants assessed x highest degree}}\right]x100 \quad (3)$$

Re-isolation of the fungus

The fungus from the diseased plant material was isolated to determine if the symptoms observed were caused by *F. oxysporum*. Likewise, the isolation from the vascular region of asymptomatic plants to determine if there was a slight infection in the basal part of the stem in any of the genotypes was performed; to demonstrate possible asymptomatic carriers (Estupiñan and Ossa, 2007).

Data analysis

An analysis of variance on the data using S.A.S 9.3 software (Statistical Analysis System, Institute Inc.) was performed. When the null hypothesis was rejected, there were differences in the variables between treatments. This was determined according to De la Cruz *et al*. (2010), who established significant differences between treatments when the upper values exceed the mean plus one standard error ($\mu+\sigma$) and highly significant when the upper values exceed the mean plus two standard errors ($\mu+2\sigma$). In this case, for disease I and S, the most outstanding genetic materials were those found below $\mu-\sigma$ or $\mu-2\sigma$, with significant or highly significant differences compared to the other genotypes.

RESULTS AND DISCUSSION

There were significant differences for plant height and stem diameter, between the genotypes assessed through sexual seed (Table 4). Genotypes 22A, 15B, 15C and Castilla showed the highest ΔPH with values between 0.34 and 0.41 cm day^{-1} in comparison with the control, indicating that the infection process caused by the fungus within the vascular system of these genotypes, generated a relatively low decrease in apical growth, which may be due to a mild or first-stage infection process corresponding to epidermal fixation or penetration and does not progress to a second stage, which corresponds to intravascular colonization (Gonzales *et al.,* 2012). Similarly, it can be considered that as a mechanism of infection, the pathogen can take two routes, initially hemibiotrophic and then necrotrophic (Perfect and Green, 2001), considering in this case that only the first one occurred.

Table 4. ANOVA mean squares for days to onset of symptoms (DOS), plant height (ΔPH) and stem diameter (ΔSD), evaluated in 10 lulo genotypes derived from sexual seeds inoculated with *F. oxysporum* under greenhouse conditions.

FV	GL	ΔPH	ΔSD	DOS
Repetition	4	0.0006 ns	0.00003 ns	3.56 ns
Genotype	10	0.03 **	0.00001 *	976.61 **
Error	40	0.0050	0.000005	7.97
CV(%)		24.43	24.45	24.11
R^2(%)		59.06	39.56	96.84

*, **=significant differences at $P<0.05$ and $P<0.01$, respectively; ns=non-significant; CV=coefficient of variation; R^2=coefficient of determination.

Genotype 42A showed the lowest average ΔPH with 0.14±0.06 cm day^{-1} with a more severe intravascular colonization process and affecting the apical growth (Table 5).

For ΔSD, no genotype showed a significantly different mean, except for the control that showed a highly significant difference compared to the other genotypes. The ΔSD ranged from 0.001 cm day^{-1} for 35A to 0.09 cm day^{-1} for 15C, 19A, 22A, 36B, and 42A (Table 5). In 35A and Castilla, the values of ΔSD indicates that the infection process is compromising the normal development of the stem by blocking vascular ducts. According to González *et al.* (2012), these results

showed that exist a colonization of the mycelium into the xylem and the establishment of the fungus in the plant via this tissue. This invasion through the vascular bundles causes symptoms such as dwarfism, wilt, and loss of turgor since nutrient and water transport are affected by the colonization of this pathogen, This coincides with the statements of Chekali *et al.* (2011) and Cruz *et al.* (2011), who affirmed that as a typical response to the fungus, metabolic and growth functions are altered in plants and that according to their evolution and aggressiveness, which vary by their age, their susceptibility to the pathogen and environmental conditions, they can even cause their death. The results obtained are also corroborated with similar works carried out by Clavijo (2014), which by means of pathogenicity tests with *Fusarium oxysporum* in the chili bell pepper crop was demonstrated that in addition to the susceptibility of this crop to the pathogen, it influenced in the growth variables such as height and stem diameter at the end of its evaluation.

Table 5. Averages of the variables plant height (ΔPH) and stem diameter (ΔSD) in 10 genotypes of lulo obtained through sexual seed.

Genotype	PH	SD
15B	0.34*	0.008
15C	0.34*	0.009
16A	0.29	0.007
19A	0.31	0.009
22A	0.36*	0.009
35A	0.18	0.001
36B	0.28	0.009
37A	0.30	0.008
37B	0.29	0.008
42A	0.14	0.009
Castilla	0.41**	0.003
Control (DW)	0.63**	0.011**
µg	0.28	0.009
σg	0.06	0.001
µg + σg	0.34	0.010
µg + 2σg	0.40	0.010

*, **=significant differences at $P<0.05$ and $P<0.01$, respectively; DW=distilled water; µg=overall average; σg=standard deviation

For DOS variable, there were highly significant differences between genotypes (Table 4). Among the 22 genotypes, 44% showed a disease incubation period between 7 and 14 days. These results differ from those reported by Manangón *et al.* (2015), Maya and Lagos (2011) and Narváez and Zambrano (2006), who obtained incubation averages between 21 and 46 days in different accessions of the *Lasiocarpa* section under controlled conditions and can be explained by the difference in the infective capacity of *F. oxysporum* isolates and the resistance or susceptibility of the genetic materials evaluated (González *et al.,* 2012).

Genotypes 15C, 36B, HSF1, HSF10, HSF36 and control did not show signs of vascular and root wilt during the trial period (Table 6). However, the intensity of infection caused by *F. oxysporum* is often visually diagnosed by the typical symptoms of the disease, but it is not proportional with internal infection processes such as vascular colonization and it is necessary to evaluate organs such as root and stem to determine if an infectious process exists (Ríos *et al.,* 2018). To confirm these findings, we re-inoculated with isolates Fo1, Fo2, Fo16, and Fo19 after three months of the assessment; there was not visible disease symptoms,

vascular colonization processes or any type of root necrosis.

Genotypes 16A, 19A, 42A, HSF7 and HSF25 showed the lowest averages, with the onset of symptoms at seven days post-inoculation (DPI), as demonstrated by chlorotic leaves and loss of turgor. The highest average was obtained by HSF15 with 64.4 DPI, followed by 37B with 42 DPI, and HSF4 with 37.8 DPI (Table 6).

It is worth mentioning that Ortiz (2011) reported incubation periods of 8 to 10 days for plants inoculated with *F. oxysporum* and subjected to excess water stress in another Solanaceae (*Physalis peruviana*) and 18 to 24 days for plants also inoculated under normal humidity conditions. These data added to those reported by Clavijo (2014), who found the appearance of symptoms such as yellowing, necrosis and defoliation in about six days after inoculation with the pathogen, suggest variability in the first place, with respect to the environmental influence on the expression of the pathogen and secondly, to the virulence of the inoculated strain. This aspect confirms the importance of the response of the genotypes that were re-inoculated, since their initial behavior did not vary.

Table 6. Average days to onset of symptoms (DOS) in 22 lulo genotypes inoculated with *Fusarium oxysporum* under greenhouse conditions.

Genotypes	DOS	Genotypes	DOS
15B	35.0	HSF15	64.4
15C	WS**	HSF22	18.2
16A	7.0	HSF25	7.0
19A	7.0	HSF28	11.2
22A	12.6	HSF29	8.4
35A	9.8	HSF36	WS**
36B	WS**	HSF45	14.0
37A	8.4	Castilla	WS
37B	42.0	La Selva	WS
42A	7.0	Control (DW)	WS
HSF1	WS**	**µg**	14.13
HSF2	14.0	**σg**	16.42
HSF4	37.8	**µg + σg**	0.00
HSF7	7.0	**µg + 2σg**	0.00
HSF10	WS**		

*. **=significance levels at $P<0.05$ and $P<0.01$, respectively; DW=distilled water; µg=overall mean; σg=standard deviation; WS=without symptoms.

Incidences (I) of vascular wilt were between 0 and 100%. Specifically, 77.3% of the genotypes (17 genotypes) showed I of 100% and 22.7% showed 0% incidences (5 genotypes). Genotypes 15C, 36B, HSF1, HSF10 and HSF36 displayed 0% I and S; thus, demonstrating resistance to isolate Fo15 and to the mix of isolates Fo1, Fo2, Fo16 and Fo19. These genotypes could be used in genetic improvement programs, evaluated as parents in different environments to determine their genetic potential and adaptation to the producing areas of the department of Nariño, based on the fact that the progress of these programs is more efficient when there is an adequate characterization of the attributes of interest in a population of a given species (Morillo *et al.,* 2019).

For the control of this disease, numerous practices and activities have been reported that are generally

based on the use of agrochemicals with their economic and environmental implications. That is the reason why the genetic improvement of resistance as a form of effective control for this disease has allowed to develop increasingly efficient strategies (García-Velasco *et al.,* 2020). In this regard, it is worth considering that the *Fusarium* genus, due to its great variability, is one of the most difficult to manage of all fungal groups (Dean *et al.,* 2012). Thus, the use of resistant varieties could reduce the incidence of the disease and for this purpose it is necessary to have genotypes within the species of interest that are favorable in this aspect (Horinouchi *et al.,* 2011). This situation confirms the importance of identifying the genotypes presented here as candidates for genetic improvement programs.

In contrast, genotypes 42A, 35A, 16A, HSF2, HSF7, HSF25 and HSF45 showed I of 100% and S between 72% and 88% (Figures 1 and 2), being highly susceptible to pathogen, with symptoms of generalized chlorosis, necrosis of new leaves, necrosis in main leaves, and loss of turgor. These results are consistent with

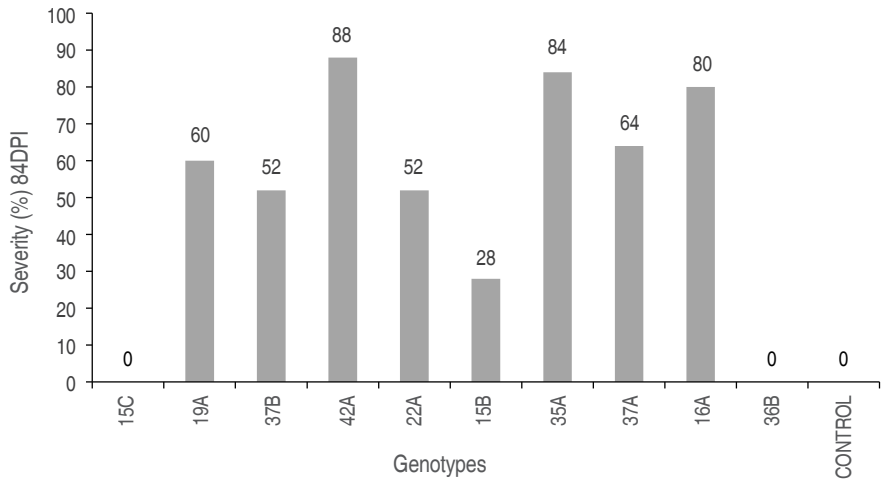

Figure 1. Average severity (%) at 84 DPI in 10 lulo genotypes obtained by sexual seed.

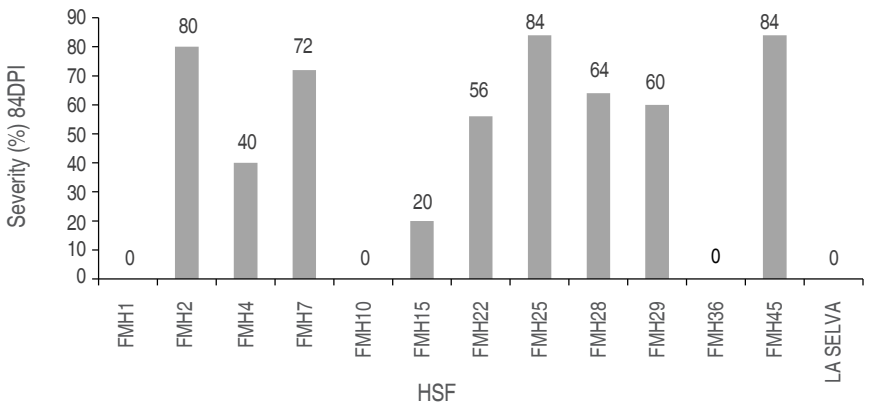

Figure 2. Average severity (%) at 84 DPI in 12 half sibling families (HSF) of lulo propagated by stakes.

those obtained by Narváez and Zambrano (2006) and Gallardo (2005), who evaluated different genotypes of *S. quitoense* and between 9 and 11 week of evaluation reached the highest level of severity in 90% of their seedlings and the remaining 10% presented low levels as chlorosis and flaccidity in the lower leaves. On the other

hand, it is also worth mentioning the work of Arizala *et al.* (2011), who evaluated the response to *F. oxysporum* of wild species of lulo, in which they highlighted low incidence for *Solanum hirtum* and *Solanum marginatum*, the former with physical compatibility in the use of grafts with *S. quitoense*, which is the reason why, in addition to those mentioned, it is possible to think about interspecific improvement processes for the study area.

Genotypes HSF15 and 15B showed S of 20% and 28%, respectively, with slight chlorosis in new leaves and a slight delay in growth. Also, these genotypes were re-inoculated with the mix of Fo1, Fo2, Fo16, and Fo19 obtaining S of 30% and 33%, finding that the two genotypes survived during the trial period, suggesting tolerance or a resistance mechanism different of the other genotypes. According to Forero *et al.* (2015), this expression of tolerance can be due to physiological factors, such as an increased concentration of chlorophyll in leaves or an increased number of new leaves or sprouts, which promote the emission and opening of floral buds or increase nutrient uptake. Also, these two genotypes, must be considered for evaluation in different environments to determinate his agronomic potential.

CONCLUSIONS

Regarding the growth variables evaluated, genotypes 22A, 15B and 15C stood out for showing the greatest increase in plant height over the susceptible Castilla control, which also, with respect to the increase in stem diameter, showed a lower value than the other genotypes, thus indicating the favorable behavior of the populations evaluated with respect to the presence of the disease.

Based on their differential response and with respect to the other genotypes in terms of periods of observation of symptoms, incidence and severity of the disease, genotypes 15C, 36B, HSF1, HSF10 and HSF36 did not show symptoms related to the inoculation of isolates Fo15 of *F. oxysporum* and the mixture of four other isolates, even after re-inoculation. This aspect is relevant for the genetic improvement of the species, since these could form the basis of a program based on resistance to *Fusarium oxysporum* for the department of Nariño, an evaluation that logically should be developed in an integral framework with other variables of productivity and agronomic behavior under cultivation conditions.

ACKNOWLEDGMENTS

The authors thank the Universidad Nacional de Colombia Palmira Campus, to the Grupo de Investigación en Producción de Frutales Andinos of the Universidad de Nariño and Sistema General de Regalías for funding this study and for allowing the completion of the Master's work that led to this article.

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Alkaline solution as a control of *Botrytis cinerea, Rhizopus stolonifer, Salmonella* spp. and *Escherichia coli* growth in strawberry (*Fragaria* x *ananassa*)

UNIVERSIDAD NACIONAL DE COLOMBIA

Soluciones alcalinas como control del crecimiento de *Botrytis cinerea, Rhizopus stolonifer, Salmonella* spp. y *Escherichia coli* en fresa (*Fragaria* x *ananassa*)

https://doi.org/10.15446/rfnam.v74n2.90319

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ABSTRACT

Keywords:
Growth inhibition
Immersion
In vitro
pH
Zone of inhibition

Post-harvest treatments of fruits and vegetables can help to reduce the attack of microorganisms especially the presence of pathogenic microorganisms. Alkaline water solutions were used to control of the growth of *Botrytis cinerea, Rhizopus stolonifer, Salmonella* spp. and *Escherichia coli* in strawberry (*Fragaria x ananassa*). Strawberries were inoculated with the microorganisms and afterwards were immersed in alkaline solutions of pH 11, 12 and 13. *In vitro* microbiological analyses were used to evaluate the presence of the microorganisms after fruit immersion in alkaline solutions, while the disc diffusion method was used to study the inhibition of microorganism growth. According to the results, alkaline solutions at pH 13 can be utilized to control *Botrytis cinerea* and *Rhizopus stolonifer* in strawberries. The immersion of strawberries in alkaline solutions at pH 13 for 60 min allowed to control *in vitro* development of *Salmonella* spp. and *Escherichia coli*.

RESUMEN

Palabras clave:
Inhibición del crecimiento
Inmersión
In vitro
pH
Zona de inhibición

Los tratamientos poscosecha de frutas y hortalizas pueden ayudar a reducir el ataque de microorganismos, en especial, la presencia de microorganismos patógenos. El presente trabajo utilizó soluciones alcalinas como control de *Botrytis cinerea, Rhizopus stolonifer, Salmonella* spp. y *Escherichia coli* en fresa (*Fragaria x ananassa*). Las fresas fueron inoculadas con los microorganismos y posteriormente sumergidas en soluciones alcalinas de pH 11, 12 y 13. Se utilizaron análisis microbiológicos *in vitro* para evaluar la presencia de los microorganismos después del proceso de inmersión de la fruta en soluciones alcalinas y para estudiar la inhibición del crecimiento de los microorganismos se utilizó el método de difusión en disco. De acuerdo con los resultados, se pueden utilizar soluciones alcalinas a pH 13 para controlar *Botrytis cinerea* y *Rhizopus stolonifer* en fresas. La inmersión de las fresas en soluciones alcalinas a pH 13 por un tiempo de 60 min permitió controlar el desarrollo *in vitro* de *Salmonella* spp. y *Escherichia coli*.

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Received: January 26, 2021; Accepted: March 08, 2021
Rev. Fac. Nac. Agron. Medellín 74(2): 9615-9619. 2021

ISSN 0304-2847 / e-ISSN 2248-7026

FAO estimates that 14% of the world's food is lost from post-harvest up to (but not including) the retail level (FAO, 2019). 25% of roots, tubers and oil-bearing crops are lost, followed by fruits and vegetables (22%), meat and animal products (12%) and cereals and pulses (9%). The most of the losses are due to microbiological and physiological deterioration as well as mechanical damage during harvesting, transportation and marketing stages.

Ecuador is an important world producer of fruits and vegetables. Among fruits, strawberry crop has been developed in Ecuador for the last years (Parra, 2018), with a monthly production of 300000 t. Microorganisms like *Botrytis cinerea* and *Rhizopus stolonifer* generate post-harvest losses of strawberry (Alcántara, 2009; Becerra *et al*., 2013). Additionally, this fruit is a carrier of some foodborne pathogens, e.g., *Escherichia coli* and *Salmonella spp.* (Carrasco and Caro, 2017). Therefore, to ensure the quality of strawberries, it is necessary to minimize the presence of pathogenic microorganisms that, at the same time, may affect consumer health (García-Robles *et al*., 2017).

There are various methods to reduce the microbiological load on the surface of fruits and vegetables. In general, the methods are based on physical processes such as mechanical removal, heat treatment, irradiation, and chemical methods. The use of an alkaline pH to control pathogenic microorganisms in food has not been widely studied. One of the reasons could be that most foods have a pH below 7. There are exceptions such as the lutefisk, an ancient tradition in Norway, Sweden and Finland of a fish prepared in lye, with a pH up to 12 (Lunestad *et al*., 2018).

In general, bacteria have an optimal growth pH close to neutrality; while fungi have a wider pH range, such as *B. cinerea*, which germinates in a pH range of 3 to 7 (Martínez and Moreno, 2008). There is a group of microorganisms, called alkaliphiles, that is developed at pH greater than 8, commonly between 9 and 10. These microorganisms are found in highly alkaline environments, such as soda lakes and carbonated soils (Lunestad *et al*., 2018).

Based on the previous information, the aim of this work was to evaluate alkaline solutions (pH 11, 12 and 13) via *in vitro* against growth of *B. cinerea* and *R. stolonifer* in strawberry. Additionally, a combination of alkaline solutions (pH 11, 12 and 13) and immersion times (20, 40 and 60 min) was used to inhibit the growth of *Salmonella* spp. and *E. coli* in strawberry.

MATERIALS AND METHODS

Strawberries (*Fragaria x ananassa*) were purchased in the central market of Manta city, Ecuador. Strawberries with an approximate weight of 20 g each, with no mechanical damage and with a maturity degree of 4, on a scale of zero to six, were chosen (NTC 4103, 1997) and washed with distilled water.

A total of 72 strawberries were used for microbiological analyses of *Salmonella* and *E. coli,* whereas 24 strawberries were used for *B. cinerea* and *R. stolonifer*. Two types of completely randomized designs were used. A unifactorial design to study of the effect of pH as a control of *B. cinerea* and *R. stolonifer*, where the independent variable was the pH at 3 levels (11, 12 and 13) and the dependent variables were microbial counts as CFU and the inhibitory effect against *B. cinerea* and *R. stolonifer*. A two-factor design was used to study the effect of pH as a control of *Salmonella* spp. and *E. coli*, being the independent variables pH (11, 12 and 13) and immersion time (20, 40 and 60 min) and the dependent variables were microbial counts as CFU and the inhibitory effect against *Salmonella* spp. and *E. coli* as mm of inhibition zone.

Control of *B. cinerea* and *R. stolonifer* by immersion in alkaline solutions

Strawberries were inoculated at 10^4 CFU mL^{-1} with *B. cinerea* and 10^5 CFU mL^{-1} with *R. stolonifer* (Camacho and Nieto, 2017). Sodium hydroxide solutions pH of 11, 12 or 13 were prepared by adding and dissolving NaOH in distilled water, under constant stirring, until the desired pH was reached. Afterwards, the fruits were placed in NaOH solutions pH 11, 12 or 13 and immediately were rinsed with distilled water. Strawberry surface swabbing was performed for microbiological analysis. Microbial growth was reported as CFU of *B. cinerea* and *R. stolonifer*, according to the methodology described by "Norma Técnica Ecuatoriana" NTE INEN 1529-10:2013 (INEN, 2013). All analyses were performed in triplicate.

Control of *Salmonella* spp. and *E. coli* by immersion in alkaline solutions

Strawberries were inoculated at 10^6 CFU mL^{-1} with both *Salmonella* and *E. coli* (Ledesma *et al*., 2018). Afterwards, the fruits were immersed in alkaline solutions pH 11, 12 or 13 during 20, 40 or 60 min. A strawberry surface sampling was performed (previously described) and microbial growth was reported as CFU.

Inhibition of the growth of *B. cinerea, R. stolonifer, Salmonella* spp. and *E. coli* by alkaline solutions

Analysis of inhibition was determined according to EUCAST (2013) with slight modifications. Petri dishes were inoculated at 10^4 CFU mL^{-1} with *B. cinerea* and 10^5 CFU mL^{-1} with *R. stolonifer* (Camacho and Nieto, 2017) using Sabouraud dextrose culture medium, whereas *Salmonella* spp. and *E. coli* were inoculated both at 10^6 CFU mL^{-1} in *Salmonella-Shigella* agar (HiMedia Laboratories, India). Afterwards, Petri dishes were incubated at 37 °C for 2 days. An amount of 20 µL of alkaline solution was added to filter paper disks (Fisher Scientific Q2) of 5 mm diameter. The disks were placed in the centre of the Petri dish, previously prepared and incubated at 25 °C with both *B. cinerea* and *R. stolonifer* for 24 h and *Salmonella* spp. and *E. coli* at 37 °C for 24 h. The zones of inhibition of microorganisms growth were measured after incubation and reported as mm of inhibition. Analyses were performed in triplicate.

Statistical analysis

Data were subjected to ANOVA and the significance of the difference between means was determined by Tukey test ($P<0.05$) with InfoStat statistics software (Infostat version 2014, Argentina). All measurements were performed in triplicate.

RESULTS AND DISCUSSION

Control of *B. cinerea* and *R. stolonifer* by immersion in alkaline solutions

Results of the effect of alkaline solutions against *B. cinerea* showed differences among the three pH ($P<0.05$). The smallest zone of inhibition was obtained by a pH 11 solution, with a diameter of 8 mm, whereas the largest zone by a pH of 13 with a diameter of 11.67 mm (Table 1). An increase of pH led to 1-log reduction from pH 11 to 12. Regarding *R. stolonifer*, there was no difference on the inhibition zone among the three pH ($P<0.05$) and additionally, the increase of pH did not cause a log reduction in CFU mL^{-1}.

Table 1. Inhibition zone and CFU counting of *B. cinerea* and *R. stolonifer* by alkaline solutions.

pH	*B. cinerea*		*R. stolonifer*	
	Zone of inhibition (mm)	CFU mL^{-1}	Zone of inhibition (mm)	CFU mL^{-1}
11	8.00 a	$1.4x10^4$	11.00 a	$5.0x10^3$
12	10.00 a,b	$4.0x10^3$	11.44 a	$3.4x10^3$
13	11.67 b	$2.0x10^3$	11.00 a	$1.0x10^3$

Different letters in the same column indicate a statistically significant difference ($P<0.05$)

Similar results of inhibition were obtained by Ahlem *et al.* (2012), showing that an alkaline pH 10 gave a better inhibition of *B. cinerea* than a lower pH. Besides, Qin *et al.* (2010) showed the effectiveness of NaOH solution to control *B. cinerea* on table grapes.

Inhibition of *B. cinerea* and *R. stolonifer* in the presence of an alkaline solution could be due to a drying effect of the microorganism resulting from osmotic dehydration. In fact, salinity affects microbes via osmotic effect by drawing water out of cells which may kill microbes through plasmolysis (Oren, 1999).

Control of *Salmonella* spp. and *E. coli* by immersion in alkaline solutions

Effect of pH. There were no differences on the zones of inhibition of *Salmonella* spp. (Table 2), whereas differences were found for *E. coli* ($P<0.05$), with zones

of inhibition of 11.56 and 12.11 mm, when solutions at pH 11 and 12, respectively, were used. Smaller zones (10.33 mm) were obtained using solutions pH 13. The highest pH values led to a higher inhibition in *Salmonella* spp. In fact, salts have been used to control *Salmonella* spp. in food (Aspridou *et al*., 2018; Tiganitas *et al*., 2009). Zhou *et al.* (2011) observed that *Salmonella* suffers an initial decline in cell numbers when inoculated into a high salt concentration medium. However, when the stress is not lethal, the cells could adapt and subsequently grow under the new condition. Similar studies in sub-lethally stressful environments reported that cell populations suffered an initial loss followed by a recovery (Mellefont *et al*., 2005). Differences of zone of inhibition between pH 13 and lower pH (11 and 12) may not reflect real differences since longer times of analyses may be needed to guarantee a full recovery of cell population.

Table 2. Control of *Salmonella* spp. and *E. coli* by immersion in alkaline solutions at different pH.

pH	*Salmonella* spp. 10^6 CFU mL^{-1} Zone of inhibition (mm)	*E. coli* 10^6 CFU mL^{-1} Zone of inhibition (mm)
11	9.89 a	11.56 b
12	9.56 a	12.11 b
13	11.22 a	10.33 a

Different letters in the same column indicate a statistically significant difference (P<0.05)

Effect of immersion time. Table 3 shows that there was no growth of *Salmonella* spp. for the three immersion times in the pH 13 solution. The 20 min immersion in a pH 11 solution showed the highest CFU counting. Regarding *E. coli,* there were differences of CFU counting among different immersion times (P<0.05). Treatment of pH 13 for 20 min showed the highest CFU counting ($5.92x10^6$ CFU mL^{-1}) and pH 13 for 60 min showed no growth. Sampathkumar *et al.* (2003) showed a reduction of CFU of *Salmonella enterica* when pH was increased of 10 to 11 within 20 min of exposure to alkaline solutions, whereas Gill *et al.* (2019) observed a reduction of *Salmonella enterica* population after exposure to NaOH solution pH 11 for 2 h. Different results may be due to the use of a different strain. The difference in growth among the bacterial species examined, could be due to different strategies to cope with osmotic stress (Wood, 2007).

Table 3. Control of *Salmonella* spp. and *E. coli* by immersion in alkaline solutions of pH 11, 12 and 13 with immersion times of 20, 40 and 60 min.

***Salmonella* spp.**			***E. coli***		
pH	**Time**	**CFU mL^{-1}**	**pH**	**Time**	**CFU mL^{-1}**
13	60 min	0.00 a	13	60 min	0.00 a
12	20 min	0.00 a	12	20 min	$1.73x10^5$ b
13	40 min	0.00 a	11	20 min	$2.16x10^5$ b
13	20 min	0.00 a	11	40 min	$1.63x10^6$ c,d
12	40 min	$2.14x10^5$ b	12	40 min	$2.03x10^6$ d
12	60 min	$2.14x10^5$ b	12	60 min	$3.01x10^6$ e,f
11	40 min	$4.35x10^5$ b	13	40 min	$3.49x10^6$ f
11	60 min	$6.50x10^5$ b	11	60 min	$5.05x10^6$ g
11	20 min	$2.16x10^6$ c	13	20 min	$5.92x10^6$ h

Different letters in the same column indicate a statistically significant difference (P<0.05).

CONCLUSIONS

The present study showed that alkaline solutions at pH 13 can control the growth of *B. cinerea* and *R. stolonifer* in strawberries. The immersion of strawberries in alkaline solutions of pH 13 for 60 min inhibited completely the growth of *Salmonella* spp. and *E. coli* in strawberries. Complementary studies of dehydration of strawberries after immersion in alkaline solutions should be performed along with the use of other alkalis.

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POLÍTICA EDITORIAL

REVISTA FACULTAD NACIONAL DE AGRONOMÍA MEDELLÍN

La Revista Facultad Nacional de Agronomía Medellín (RFNA), es una publicación de la Facultad de Ciencias Agrarias de la Universidad Nacional de Colombia - Sede Medellín. Esta orientada a profesores, investigadores, estudiantes, extensionistas y a todos aquellos profesionales que crean conocimiento y articulan la ciencia y la tecnología para hacer más productivo el campo a nivel empresarial y de economía campesina.

La Revista recibe y publica, sin ningún costo, artículos en idioma inglés de investigación, revisión, reseñas, cartas al editor y editoriales.

La periodicidad de la Revista es cuatrimestral, con circulación nacional e internacional y tiene como objetivo divulgar artículos escritos en inglés, originales, inéditos y arbitrados (peer review) de carácter científico que respondan a preguntas específicas y que proporcionen soporte y pruebas a una hipótesis, en aspectos relacionados con las Ciencias Agronómicas, Zootecnia, Ciencias Forestales e Ingeniería Agrícola y de Alimentos y otras afines que contribuyan a la solución de los limitantes del agro en el trópico.

Teniendo en cuenta los criterios considerados por Colciencias, la revista acoge documentos de las siguientes tipologías:

Artículos de investigación científica y tecnológica: Documento que presenta, de manera detallada, los resultados originales de proyectos terminados de investigación. La estructura generalmente utilizada contiene cuatro partes fundamentales: Introducción, metodología (materiales y métodos), resultados y discusión, y conclusiones. La extensión máxima debe ser de 5200 palabras, excluyendo figuras, tablas, referencias. El mínimo de referencias bibliográficas sugerido es de 10 y el máximo de 30. Este tipo de artículos es arbitrado e indexado.

Artículos de revisión: Documentos producto de una investigación terminada donde se analizan, sistematizan e integran los resultados de investigaciones publicadas o no publicadas, sobre un campo en ciencia o tecnología, con el fin de dar cuenta de los avances y las tendencias de desarrollo. Se caracteriza por presentar una cuidadosa revisión bibliográfica de por lo menos 50 referencias. La extensión máxima debe ser de 6000 palabras, excluyendo figuras, tablas, referencias. Este tipo de artículos es arbitrado e indexado.

Artículos cortos: Documento breve que presenta resultados originales preliminares o parciales de una investigación científica o tecnológica, que por lo general requieren de una pronta difusión. Para todos los casos el 60% de las citas debe provenir de artículos publicados en los últimos diez años.

Los artículos deben ser presentados de acuerdo a los lineamientos establecidos en las “Instrucciones a los Autores”; quienes incumplan las normas básicas no iniciarán el proceso editorial. Se debe diligenciar el formato “Autorización para Publicación de Obras y Cesión de Derechos Patrimoniales”, el cual será suministrado por la Revista. Dicho documento es explícito en mencionar que todos los autores están informados y de acuerdo con someter el artículo a consideración de la Revista, que no hay conflictos de interés entre ellos y expresa que el contenido del manuscrito no ha sido ni será enviado para su publicación a otra Revista.

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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 deben remitir los siguientes cuatro formatos: (1) Lista de verificación de criterios editoriales para la presentación de manuscritos; (2) Autorización de publicación de manuscritos en la Revista Facultad Nacional de Agronomía Medellín, en la cual se acepta la no postulación simultánea del artículo a otras revistas u órganos editoriales y se ceden los derechos a la Revista para su difusión, este debe ser firmado por todos los autores del manuscrito; (3) Datos personales de cada autor; (4) Sugerencia de posibles pares evaluadores. Las formas de publicación son: artículos de investigación científica y tecnológica, artículos de revisión y artículos cortos. Los artículos pueden ser elaborados por profesores y/o investigadores de la Universidad Nacional de Colombia, o cualquier otra institución afín, nacional o internacional, en los temas Agropecuarios, Forestales y de Ingeniería Agrícola y de Alimentos. El manuscrito no debe exceder 5200 palabras para artículos de investigación y 6000 para artículos de revisión. Las hojas deben ser tamaño carta, escritas a interlineado doble, numeración de línea continua, letra o fuente Times New Roman o Verdana, tamaño 12 puntos, márgenes de 3 cm en la parte superior, 2 cm en la inferior y 2,5 cm en las márgenes laterales derecha e izquierda. Las tablas y figuras (es decir, los gráficos, dibujos, esquemas, diagramas de flujo, fotografías y mapas) se deben mostrar incorporadas en el texto y con numeración consecutiva (Tabla 1... Tabla n; Figura 1... Figura n, etc.). Los textos y tablas se deben presentar en el procesador de palabras MS-Word®;las tablas y los diagramas de frecuencia (barras y tortas) originales se deben suministrar en el archivo del documento y también en su original de MS-Excel®; otras figuras, como fotografías sobre papel y dibujos, se pueden enviar en original o escaneadas y ser remitidas en el formato digital de compresión JPG (o JPEG) preferiblemente con una resolución de 600 x 600 dpi (mínimo 300 dpi); es deseable que las fotografías originales sean enviadas como diapositivas. Como norma general, las tablas y figuras sólo se aceptan en blanco y negro; excepcionalmente se incluirán en color cuando sea estrictamente necesario y a juicio del Comité Editorial.

Unidades, abreviaturas y estilo

Se debe utilizar el Sistema Internacional de Unidades (SIU), y aquellas unidades específicas de mayor uso por parte de la comunidad científica. Las unidades combinadas deben usar la forma exponencial. Ejemplo: kg ha^{-1}. El significado de las abreviaturas debe citarse por extenso cuando se mencionan por primera vez en el manuscrito. El estilo de escritura debe ser absolutamente impersonal, en tiempo gramatical pasado para la introducción, los procedimientos y los resultados y presente para la discusión, evitando la conjugación de verbos en primera o tercera persona del singular o el plural.

Los números del uno al nueve se escriben en palabras, excepto cuando incluyen unidades de medida o se mencionan varios números. Ejemplo: “ocho tratamientos”, “3, 7 y 9 lecturas”, “15 kg”. Use cero antes del punto decimal. Para separar números en intervalos de uno o más años, use la letra “a”, y guión para temporadas de crecimiento. Ejemplo: Periodo 2002 a 2005; temporadas de crecimiento 1999-2000, 2000-2001.

Título y autores

El título del artículo no debe incluir abreviaturas y es obligatoria su respectiva traducción al idioma español. En lo posible, el título no debe exceder de 15 palabras y debe reflejar con precisión el contenido del documento. Cuando contenga nombres científicos de especies vegetales o animales, éstos se deben escribir con letra cursiva (itálica) en minúsculas, sólo con mayúsculas la primera letra del género y del clasificador. Debajo del título en inglés se escribe el nombre(s) y apellido(s) de los autores, sin sus respectivos títulos académicos, ni cargos laborales, en una línea horizontal y de acuerdo con su contribución en la investigación y/o preparación del artículo.

Como nota al pie de la primera página, se escribe el título de pregrado, el cargo laboral de los autores, el nombre y la ciudad de ubicación de la entidad a la cual prestan sus servicios o del patrocinador para la realización del trabajo y su respectiva dirección de correo electrónico, indicando el autor de correspondencia. Además, se debe adjuntar un resumen de la hoja de vida de los autores, donde se mencionen los artículos publicados en otras revistas.

Resumen, abstract y palabras claves

El resumen no debe exceder de 250 palabras escritas en un único párrafo. Se debe escribir en inglés y español. Debe contener en forma breve la justificación, los objetivos, los métodos utilizados, los resultados obtenidos más relevantes y las conclusiones. Es obligatorio acompañar el resumen con un máximo de seis palabras clave distintas a las utilizadas en el título. Se aceptan como palabras clave no sólo las palabras simples, sino también términos compuestos hasta de tres palabras. Deben ir escritas en minúsculas y separadas por comas.

Introducción

Puede tener o no título. Define el problema e informa sobre el estado del arte respecto al tema principal del artículo; además, señala las razones que justifican la investigación y plantea los objetivos de la misma. Es obligatorio acompañar los nombres vulgares con el nombre(s) científico(s) y la abreviatura(s) del clasificador en la primera mención dentro del texto. No se deben mencionar marcas de productos, sino su nombre genérico o químico

Materiales y métodos

En este apartado se deben describir en forma clara, concisa y secuencial, los materiales (vegetales, animales, implementos agrícolas o de laboratorio) utilizados en el desarrollo del trabajo; además, se mencionan los aspectos relacionados con la ubicación, preparación y ejecución de los experimentos. Se debe indicar el diseño seleccionado, las variables registradas, las transformaciones hechas a los datos, los modelos estadísticos usados y el nivel de significancia empleado. Evitar detallar procedimientos previamente publicados.

Resultados y discusión

Son la parte central del artículo, deben estar respaldados por métodos y análisis estadísticos apropiados. Se deben presentar de manera lógica, objetiva y secuencial mediante textos, tablas y figuras; estos dos últimos apoyos deben ser fáciles de leer, autoexplicativos y estar siempre citados en el texto. Las tablas se deben elaborar con pocas columnas y renglones. Se debe tener la precaución de incluir el nivel de significancia estadística representado por letras minúsculas del comienzo del alfabeto (a, b, c, d,...), un asterisco simple (*) para $P<0,05$, doble asterisco (**) para $P<0,01$ o triple asterisco (***) para $P<0,001$. Las investigaciones que no siguen un diseño estadístico, deben mostrar la información de manera descriptiva. Use subíndices para modificaciones, reserve superíndices para potencias o notas al pie en tablas y figuras.

La discusión: Se refiere al análisis e interpretación objetiva de los resultados, confrontándolos con los obtenidos en otras investigaciones, o con los hechos o teorías conocidos sobre el tema. Explica los resultados en particular cuando difieren de la hipótesis planteada. Destaca la aplicación práctica o teórica de los resultados obtenidos y

las limitaciones encontradas. Resalta la contribución que se hace a una determinada área del conocimiento y el aporte a la solución del problema que justifica la investigación. Finalmente, proporciona elementos que permitan proponer recomendaciones o lanzar nuevas hipótesis. No se deben hacer afirmaciones que van más allá de lo que los resultados pueden apoyar.

Conclusiones

Son las afirmaciones originadas a partir de los resultados obtenidos, deben ser coherentes con los objetivos planteados y la metodología empleada; además, expresar el aporte al conocimiento en el área temática estudiada y proponer directrices para nuevas investigaciones.

Agradecimientos

Si se considera necesario, se incluyen los agradecimientos o reconocimientos a personas, instituciones, fondos y becas de investigación, que hicieron contribuciones importantes en la concepción, financiación o realización de la investigación.

Formato de citación en el texto

- Se registra la fuente entre paréntesis, el cual debe incluir el apellido del autor y año, con coma entre autor y año. Ejemplo: (Pérez, 1995).

- Si hay más de una fecha se separarán con comas: Ejemplo: (Pérez, 1995, 1998, 2001)

- Si hay dos autores se citarán separados por la conjunción and. Ejemplo: (Gil and Ortega, 1993)

- Si hay varios trabajos de un autor publicados en un mismo año, se citarán con una letra en secuencia alfabética de los títulos, adosada al año. Ejemplo: (Gómez, 2000a, 2000b, 2000c)

- En el caso de citas con tres o más autores, es necesario mencionar en el texto el apellido del primero y reemplazar los demás por la expresión latina abreviada *et al.* (en cursiva) que significa y otros; en la referencia se deben poner los apellidos e iniciales de todos los autores. Ejemplo: (García *et al*., 2004).

- Cuando se hace referencia al autor dentro del texto, sólo se encierra el año entre paréntesis y se omite la coma que separa al autor del año. Ejemplo: (1) De acuerdo con Castañeda (2000), …; (2) Concorde con los resultados de Poveda *et al.* (2018) …

- Cuando es una cita de una cita se ponen la información de los autores citados y los autores citantes. Ejemplo: Magalhaes *et al.* (1979) expone que … (as cited in Gómez, 2004).

- Organizaciones se citan por sus siglas, en caso de no tener se cita con su nombre completo. Ejemplo: (1) (FAO, 2015), (2) (Ministerio de Agricultura y Ganadería, 2019)

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

Las referencias bibliográficas se deben ordenar alfabéticamente por el apellido del primer autor, sin numeración y sin sangría. Para citar varias publicaciones del mismo autor, se debe seguir el orden cronológico creciente; si son del mismo año, se debe seguir el orden alfabético de los títulos.

Las referencias deberán contener todos los datos que permitan su fácil localización. Las referencias se citan en el lenguaje de publicación.

En cada referencia para todos los autores cite primero el apellido, tener en cuenta que algunos autores hispanos citan sus dos apellidos, seguido de la inicial del nombre sin puntos, separando autores con coma y espacio.

Ejemplos:

Libros: Autor(es). Año. Título del libro. Edición. Casa editora, ciudad de su sede. Páginas consultadas (pp. #-#) o páginas totales (# p.). Ejemplo: Robinson A, Morrison J, Muehrcke P, Kimerling AJ and Guptill S. 1995. Elements of Cartography. Sixth edition. John Wiley and Sons, Inc., New York. 674 p.

García Rodríguez JL, Giménez Suarez MC, Ortega Pérez E, Martín Ramos B, Calderón Guerrero C. 2014. Operaciones auxiliares en repoblaciones e infraestructuras forestales. Ediciones Paraninfo SA, Madrid. 208 p.

Capítulos de libros: Autor(es). Año. Título del capítulo. Páginas consultadas (pp. # - #). En: Apellidos e iniciales de los compiladores o editores (eds.). Título del libro. Edición. Casa editora, ciudad de su sede. Páginas totales (# p.). Ejemplo: Bernal H. 1996. Capítulo 6: Evapotranspiración. pp. 112-125. En: Agrios G. (ed.). Fitopatología. Segunda edición. Editorial Limusa, México D.F. 400 p.

Bertoft E and Blennow A. 2016. Chapter 3 - Structure of potato starch. pp 57-73. In: Singh J and Kaur L. (eds.). Advances in potato chemistry and technology. Second edition. Academic Press, London. 752 p.

Artículos de revistas: Autor(es). Año. Título del artículo. Nombre completo de la revista volumen(número de fascículo): página inicial–página final. doi. Ejemplo: García S, Clinton W, Arreaza L and Thibaud R. 2004. Inhibitory effect of flowering and early fruit growth on leaf photosynthesis in mango. Tree Physiology 24(3): 387-399. doi: 10.1093/treephys/24.4.387

Ponencias en memorias de congresos, seminarios, simposios: García M. 1998. La ingeniería geotécnica y la protección del medio ambiente. pp. 65-94. En: Memorias IX Congreso Colombiano de la Ciencia del Suelo. Sociedad Colombiana de la Ciencia del Suelo, Bogotá.

High R. 2015. Plotting LSMEANS and Differences in Generalized Linear Models with GTL. In: 2015 Midwest SAS Users Group Conference Proceedings. Midwest SAS Users Group, Omaha. 9 p.

Tesis, trabajos de grado. Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín, Colombia. 78 p.

Adam M. 1992. The Impact of the Common Agricultural Policy on Agriculture in Greece (Master's thesis). Cambridge University. Cambridge, United Kingdom. 80 p.

Cita de cita, sólo se referencia la fuente consultada. Ejemplo: Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín, Colombia.

Suplemento de revista: Silva AM y Carrillo NN. 2004. El manglar de piruja, Golfito, Costa Rica: un modelo para su manejo. Revista de Biología Tropical 52 Suppl. 2: 195-201.

Citas de internet: Autor(es). Año. Título del artículo. En: Nombre(s) de la publicación electrónica, de la página web, portal o página y su URL, páginas consultadas (pp. # - #) o páginas totales (# p.); fecha de consulta. Ejemplo: Arafat Y. 1996. Siembra de olivos en el desierto palestino. In: Agricultura Tropical, http://agrotropical.edunet.es. 25 p. consulta: noviembre 2003.

Patentes: Autor(es). Año. Título. País de la patente y número. Fuente. Ejemplo: Glenn RW. 1996. Liquid personal cleansing compositions which contain soluble oils and soluble synthetic surfactants. U.S. Patent No. 6194364. Retrieved from: https://patents.google.com/patent/US6194364B1/en

PUBLISHING POLICY

REVISTA FACULTAD NACIONAL DE AGRONOMÍA MEDELLÍN

The Journal *Revista Facultad Nacional de Agronomía Medellín* (RFNA) is published by the Faculty of Agricultural Sciences of Universidad Nacional de Colombia – Medellín. It is aimed at professors, researchers and students in agronomy, animal, and forestry sciences, food and agricultural engineering, agricultural advisers and at all those professionals who create knowledge and articulate science and technology to make the field more productive at business and rural economy levels.

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The Journal is a four-monthly publication at national and international level. Its aim is to publish original, unpublished, and peer-reviewed articles of a scientific nature which respond to specific questions and provide support and testing of a hypothesis, related to agronomy, animal husbandry, forestry engineering, food and agricultural engineering, and related areas that contribute to the solution of the agricultural constraints in the tropics.

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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.

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INSTRUCTIONS TO AUTHORS

General guidelines

Papers must be sent b through the Open Journal System in the Universidad Nacional de Colombia journals web side http://www.revistas.unal.edu.co/, Will be considered only papers written in English. The four following formats must be submitted with the manuscript: (1) Editorial Criteria Checklist for Paper Submission; (2) Paper Publishing Authorization for the Revista Facultad Nacional de Agronomía Medellín, which accepts no simultaneous nomination of the article to other journals or editorial bodies, and the rights are given to the Journal for its release by the signature of all the manuscript's authors; (3) Personal information of each author; (4) Suggestion of possible peer reviewers. Publishing forms are: scientific and technological research articles, review articles, reflection articles, and short articles. Articles can be developed by professors and/or researchers at the Universidad Nacional de Colombia, or other related national or international institution, on Agricultural, Forestry, Food and Agricultural Engineering matters. Article extension must not exceed 5,200 words for research articles and 6,000 words for reviews. The manuscript must be letter-size sheets, line spacing double, continuous line number 12 point Times New Roman or Verdana font, 3 cm margin at the upper, 2 cm in the lower, 2.5 cm on the left and right side margins. Tables and figures (i.e, graphics, drawings, diagrams, flowcharts, photographs and maps) should be shown on separate sheets and numbered consecutively (Table 1 ... Table n, Figure 1…Figure n, etc.). Texts and tables should be submitted in MS-Word® word processor, original tables and diagrams of frequency (bar charts and pie charts) must be supplied in manuscript file and in its original MS-Excel®; other figures, such as photographs on paper and drawings, can be sent in original or scanned and sent in digital format compression JPG (or JPEG), preferably with a resolution of 600 x 600 dpi (300 dpi at least); original photographs are suggested to be sent as slides. As a general rule, tables and figures are only accepted in black and white. Color figures will be exceptionally accepted when strictly necessary and under discretion of the Editorial Board.

Units, abbreviations and style

International System of Units (SI), and those specific units of greater use by the scientific community must be used. When required must be used the exponential form. Example: kg ha^{-1}. The meaning of abbreviations should be cited in full when first mentioned in the manuscript. The writing style should be totally impersonal. Introduction, procedures and results should be written in grammatical past tense. Discussion should be written in grammatical present tense, avoiding the conjugation of verbs in first or third person singular or plural.

The numbers from 1 to 9 are written in words, except when they include units of measure or several numbers are listed. Example: “eight treatments”, “3,7 and 9 readings”, “15 kg”. Use zero before the decimal point. To separate numbers in intervals of one to two years, use the letter “a” and hyphen for growing seasons. Example period 2002 to 2005, growing seasons 1999-2000, 2000-2001.

Title and authors

The article should not include abbreviations and its translation into English is required. As far as possible, the title should not exceed 15 words and must accurately reflect the paper content. When the article contains scientific names of plants or animals, they should be written in italics in lower case, only the first letter of gender and classifier should be capital. Under the title in English the author or authors' name (s) and surname (s) is /are written, without academic degrees or job positions, in a horizontal line according to the contribution to research and / or preparation of the article.

As a footnote on the first page, write the title of undergraduate, authors' job positions, the name and city location of the entity to which they serve, or the sponsors for the research work and their respective email address. In addition, a summarized authors' résumé including reference to the articles published in other magazines should be attached.

Abstract and key words

The abstract should not exceed 250 words written in a single paragraph. It must be written in English and Spanish. It should contain in brief the justification, aims, methods used, the most relevant results, and conclusions. It is required to accompany the abstract with a maximum of six key words, translated into English, different from those used in the title. Single words as well as compound terms of up to three words are accepted as key words. They must be written in lowercase, separated by commas.

Introduction

It may or not have a title. It defines the problem and reports on the state of the art on the main subject of the article, it also points out the reasons for the research and sets out its aims. It is required to accompany common names with the corresponding scientific name (s) name and abbreviation (s) of the classifier at the first mention in the text. Brands must not be mentioned but the generic or chemical name.

Materials and methods

In this section, materials (crops, livestock, agricultural or laboratory implements) used in the development of work should be clearly, concisely and sequentially described. Aspects related to the location, preparation and execution of experiments should also be mentioned. The selected design, the recorded variables, the changes made to data, the statistical models used and the significance level used should be indicated. Authors must avoid detailing procedures previously published.

Results

They are the central part of the article and must be supported by appropriate statistical methods and analysis. They should be presented in a logical, objective and sequential way through texts, tables and figures; the latter two supports should be easy to read, self- explanatory and always quoted in the text. The tables should be composed by few columns and rows. Care should be taken to include the statistical significance level represented by lowercase letters of the beginning of the alphabet (a, b, c, d,…), a single asterisk (*) for $P<0.05$, double asterisk (**) for $P<0.01$ or triple asterisk (***) for $P<0.001$. Researches that do not follow a statistical design should display the information in a descriptive way. Use subscripts to modifications, reserve superscripts for potencials or footnotes in tables and figures.

Discussion

It refers to the analysis and objective interpretation of results, comparing them with those obtained in other research, or with known facts or theories on the subject. It explains the results, especially when they differ from the stated hypothesis. It emphasizes the practical or theoretical application of the obtained results and constraints encountered. Discussion also highlights the contribution that is made to a particular area of knowledge and to the solution of the problem that justifies the research. Finally, it provides elements that allow making recommendations or launching new hypotheses. Statements that go beyond what the results may support should be avoided.

Conclusions

Conclusions are assertions arising from the obtained results. They should be consistent with the objectives stated and the methodology used. They should also express the contribution to knowledge in the studied subject area and propose guidelines for further researches.

Acknowledgements

If necessary, acknowledgements or recognitions to individuals, institutions, funds and research grants that made important contributions in the design, financing or carrying out of the research are included.

Citing in-text format

- Citations in the text should be in parenthesis and include author's surname and year, with comma in-between. Example: (Pérez, 1995).

- If more than one date, they are separated by commas: Example: (Pérez, 1995, 1998, 2001).

- If there are two authors, they will be separated by the conjunction and. Example: (Gil and Ortega, 1993)

- If there are several works of an author published in the same year, they will be cited with a letter in alphabetical sequence of titles, adjacent to year. Example: (Gómez, 2000a, 2000b, 2000c)

- For citations with three or more authors, it is necessary to mention in the text the surname of the first author and replace the others by the Latin expression *et al.* (in italics), which means and others. All authors should be mentioned in the reference. Example: (García *et al.*, 2004)

- When the author is referenced within the text, only the year is enclosed in parentheses, and the comma that separates the author from the year is omitted. Example: (1) According to Castañeda (2000), ...; (2) In accordance with the results of Poveda *et al.* (2018), ...

- When an indirect source is cited, the information of the cited authors and the citing authors are placed. Example: (Magalhaes *et al.* (1979) state that ... (as cited in Gómez, 2004).

- Organizations are cited by their initials; in case they do not have their full name is used. Example: (1) (FAO, 2015), (2) (Ministerio de Agricultura y Ganadería, 2019)

References

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

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

References should contain all the data allowing to its easy location. The titles of the papers, the surnames of the authors and the names of journals must be referenced and cited in their original language.

Examples:

For books: Author(s), Year. Book title, Edition, Publisher, Place of publication. Pages consulted (pp. #-#) or total pages.Example: Robinson A, Morrison J, Muehrcke P, Kimerling AJ and Guptill S. 1995. Elements of cartography. Sixth edition. John Wiley and Sons, Inc., New York. 674 p.

García Rodríguez JL, Giménez Suarez MC, Ortega Pérez E, Martín Ramos B, Calderón Guerrero C. 2014. Operaciones auxiliares en repoblaciones e infraestructuras forestales. Ediciones Paraninfo SA, Madrid. 208 p.

For book chapters: Author(s). year. Chapter title. pages consulted (pp. # - #). In: Surnames and names of the editors or publishers (eds.). book title. Edition. Publisher, place of publication. total pages (# p.). Example: Bertoft E and Blennow A. 2016. Chapter 3 - Structure of potato starch. pp 57-73. In: Singh J and Kaur L. (eds.). Advances in potato chemistry and technology. Second edition. Academic Press, London. 752 p.

Beral H. 1996. Capítulo 6: Evapotranspiración. pp. 112-125. En: Agrios G. (ed.). Fitopatología. Segunda edición. Editorial Limusa, México D.F. 400 p.

For journals: Author(s). year. Article title. journal full name volume(number): initial page-final page. Example: García S, Clinton W, Arreaza L and Thibaud R. 2004. Inhibitory effect of flowering and early fruit growth on leaf photosynthesis in mango. Tree Physiology 24(3): 387-399. doi: 10.1093/treephys/24.4.387

Presentations in Memoirs of Congresses, seminars and symposia: García M. 1998. La ingeniería geotécnica y la protección del medio ambiente. pp. 65-94. En: Memorias IX Congreso Colombiano de la Ciencia del Suelo. Sociedad Colombiana de la Ciencia del Suelo, Bogotá.

High R. 2015. Plotting LSMEANS and Differences in Generalized Linear Models with GTL. In: 2015 Midwest SAS Users Group Conference Proceedings. Midwest SAS Users Group, Omaha. 9 p.

Theses and dissertations: Adam M. 1992. The impact of the common agricultural policy on agriculture in Greece (Doctoral dissertation). Cambridge University. Cambridge, United Kingdom. 80 p.

Gómez C. 2004. Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín. Colombia. 78 p.

Citation of a citation, list the secondary source in your reference list: Example: Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín, Colombia. 78 p.

Journal Supplement: Silva AM y Carrillo NN. 2004. El manglar de piruja, Golfito, Costa Rica: un modelo para su manejo. Journal of Tropical Biology 52 Suppl. 2: 195-201.

For internet citations: Author (s), year. Article. In: electronic publishing Name (s), the web page, portal or page name and its URL, pages consulted (pp. # - #) or total pages (# p.), date of consultation. Example: Arafat Y. 1996. Siembra de olivos en el desierto palestino. En: Tropical Agriculture, http://agrotropical.edunet.es. 25 p.; accessed: November 2003.

Patents: Author(s). Year. Title. Patent country and number. Retrieved from. Example: Glenn RW. 1996. Liquid personal cleansing compositions which contain soluble oils and soluble synthetic surfactants. U.S. Patent No. 6194364. Retrieved from: https://patents.google.com/patent/US6194364B1/en

ÉTICA EN LA PUBLICACIÓN CIENTÍFICA Y ACUERDO SOBRE POSIBLES MALAS PRÁCTICAS

La revista Facultad Nacional de Agronomía espera y verificará que los autores, revisores, editores y en general la comunidad académica y científica involucrada en nuestro proceso editorial, sigan estrictamente las normas éticas internacionales requeridas en el proceso de edición.

La revista Facultad Nacional de Agronomía sigue las normas éticas presentes en el COPE Best Practice Guidelines for Journal Editors y por el International Standars for Editors and Authors publicado por Committee on Publication Ethics.

Los autores deben evitar incurrir al plagio de la información. La revista define los siguientes lineamientos, criterios y recomendaciones sobre la ética en la publicación científica:

1. Criterios generales[1]

1.1. Los artículos deben contener suficiente detalle y referencias que permitan replicar o rebatir el estudio.

1.2.Declaraciones fraudulentas o deliberadamente inexactas constituyen un comportamiento poco ético.

1.3. Si el estudio incluye productos químicos, procedimientos o equipos que tienen cualquier riesgo inusual inherente a su uso, el autor debe identificar claramente estos en el artículo.

1.4. Si el estudio implica el uso de animales o de seres humanos, el autor debe asegurarse que el artículo contenga una declaración que haga explícito que se realizaron todos los procedimientos de conformidad con las leyes y directrices institucionales.

1.5. Se deben respetar los derechos de privacidad de los seres humanos.

2. Autoría[2]

Criterios:

2.1. Un "autor" es la persona que ha hecho una contribución intelectual significativa al artículo, por lo tanto, todas las personas nombradas como autores deben reunir los requisitos de autoría, y todos aquellos que los reúnan deben ser mencionados de forma explícita.

2.2. Se deben cumplir colectivamente tres criterios básicos para ser reconocido como autor:

a) Contribución sustancial a la concepción y diseño, adquisición de datos, análisis e interpretación del estudio.

b) Redacción o revisión del contenido intelectual.

c) Aprobación de la versión final.

2.3. El orden de la autoría debe ser una decisión conjunta de los coautores.

2.4. Las personas que participen en un estudio pero que no se ajusten a los criterios de autoría deben aparecer como "Colaboradores" o "Personas reconocidas".

2.5. Hay tres tipos de autorías que se consideran inaceptables: autores "fantasma", que contribuyen sustancialmente pero no son reconocidos (a menudo pagados por promotores comerciales); autores "invitados", que no hacen ninguna contribución discernible pero se nombran para aumentar las posibilidades de publicación; y autorías "honorarias", que se basan únicamente en una afiliación tenue con un estudio.

Recomendaciones:

2.6. Antes de iniciar la investigación se recomienda documentar la función y la forma como se reconocerá la autoría de cada investigador.

2.7. No se debe mentir sobre la participación de una persona en la investigación o publicación, si su contribución se considerada "sustancial" se justifica la autoría, bien sea como coautor o colaborador.

2.8. No se debe asignar una autoría sin contar con el consentimiento de la persona.

2.9. Todas las personas nombradas como autores deben reunir los requisitos de autoría, y todos aquellos que reúnan los requisitos deben aparecer como autores o contribuidores.

2.10. Algunos grupos colocan los autores por orden alfabético, a veces con una nota para explicar que todos los autores hicieron contribuciones iguales al estudio y la publicación.

3. Cambios en la autoría[3]

Criterios:

3.1. Hace referencia a la adición, supresión o reorganización de los nombres de autor en la autoría de un artículo aceptado.

3.2. Las peticiones de añadir o eliminar un autor, o para reorganizar los nombres de los autores, deben ser enviados por el autor correspondiente del artículo aceptado, y deben incluir:

a) La razón por la cual debe ser añadido o eliminado, o los nombres de los autores reorganizado.

b) La confirmación por escrito (e-mail) de todos los autores que están de acuerdo con la adición, supresión o reorganización. En el caso de adición o eliminación de los autores,esto incluye la confirmación de que el autor sea añadido o eliminado.

4. Conflicto de intereses[4]

Criterios:

4.1. Cuando un investigador o autor, editor tenga alguna opinión o interés financiero/personal que pueda afectar su objetividad o influir de manera inapropiada en sus actos, existe un posible conflicto de intereses. Este tipo de conflictos pueden ser reales o potenciales.

4.2. Los conflictos de intereses más evidentes son las relaciones financieras, como:

a) Directas: empleo, propiedad de acciones, becas, patentes.

b) Indirectas: honorarios, asesorías a organizaciones promotoras, la propiedad de fondos de inversión, testimonio experto pagado.

4.3. Los conflictos también pueden existir como resultado de relaciones personales, la competencia académica y la pasión intelectual. Por ejemplo, un investigador que tenga:

a) Algún tipo de interés personal en los resultados de la investigación.

b) Opiniones personales que están en conflicto directo con el tema que esté investigando.

Recomendaciones:

4.4. Revelar si se está en algún conflicto real o potencial de intereses que influya de forma inapropiada en los hallazgoso resultados del trabajo presentado, dentro de los tres (3) años de haber empezado el trabajo presentado que podría influir indebidamente (sesgo) el trabajo.

4.5. Revelar el papel de un promotor (o promotores) del estudio, si los hubiere, en el diseño del estudio, en la recopilación, análisis e interpretación de los datos, en la redacción del informe y en la decisión de presentar el documento para su publicación.

4.6. Los investigadores no deben entrar en acuerdos que interfieran con su acceso a todos los datos y su capacidad de analizarlos de forma independiente, y de preparar y publicar los manuscritos.

4.7. Al presentar un documento, se debe hacer una declaración (con el encabezamiento "Papel que ha tenido la fuente de financiación") en una sección separada del texto y colocarse antes de la sección "Referencias".

4.8. Algunos ejemplos de posibles conflictos de intereses que deben ser revelados, incluyen: empleo, consultoría, propiedad de acciones, honorarios, testimonio experto remunerado, las solicitudes de patentes / registros y subvenciones u otras financiaciones.

4.9. Todas las fuentes de apoyo financiero para el proyecto deben ser revelados.

4.10. Se debe describir el papel del patrocinador del estudio.

5. Publicación duplicada[5]

Criterios:

5.1. Los autores tienen la obligación de comprobar que su artículo sea basado en una investigación original (nunca publicada anteriormente). El envío o reenvío intencional de su trabajo para una publicación duplicada se considera un incumplimiento de la ética editorial.

5.2. Se produce una publicación duplicada o múltiple cuando dos o más artículos, sin hacerse referencias entre sí, comparten esencialmente las

mismas hipótesis, datos, puntos de discusión y/o conclusiones. Esto puede ocurrir en diferentes grados: Duplicación literal, duplicación parcial pero sustancial o incluso duplicación mediante parafraseo.
5.3. Uno de los principales motivos por los que la publicación duplicada de investigaciones originales se considera no ético es porque puede dar lugar a una "ponderación inadecuada o a un doble recuento involuntario" de los resultados de un estudio único, lo que distorsiona las pruebas disponibles.

Recomendaciones:

5.4. Los artículos enviados para su publicación deberán ser originales y no deberán haberse enviado a otra editorial. En el momento del envío, los autores deberán revelar los detalles de los artículos relacionados (también cuando estén en otro idioma), artículos similares en prensa y traducciones.
5.5. Aunque un artículo enviado esté siendo revisado y no conozca el estado, espere a que la editorial le diga algo antes de ponerse en contacto con otra revista, y sólo si la otra editorial no publicará el artículo.
5.6. Evite enviar un artículo previamente publicado a otra revista.
5.7. Evite enviar artículos que describan esencialmente la misma investigación a más de una revista.
5.8. Indique siempre los envíos anteriores (incluidas las presentaciones de reuniones y la inclusión de resultados en registros) que pudieran considerarse una publicación duplicada.
5.9. Evite escribir sobre su propia investigación en dos o más artículos desde diferentes ángulos o sobre diferentes aspectos de la investigación sin mencionar el artículo original.
5.10. Se considera manipulador crear varias publicaciones a raíz de la misma investigación.
5.11. Si desea enviar su artículo a una revista que se publica en un país diferente o en un idioma diferente, pregúntaselo a la editorial si se puede hacer esto.
5.12. En el momento del envío, indique todos los detalles de artículos relacionados en un idioma diferente y las traducciones existentes.

6. Reconocimiento de las fuentes

Criterios:

6.1. Los autores deben citar las publicaciones que han sido influyentes en la determinación de la naturaleza del trabajo presentado.
6.2. Información obtenida de forma privada, no debe ser usada sin explícito permiso escrito de la fuente.
6.3. La reutilización de las tablas y / o figuras requiere del permiso del autor y editor, y debe mencionarse de manera adecuada en la leyenda de la tabla o figura.
6.4. La información obtenida en el transcurso de servicios confidenciales, tales como manuscritos arbitrales o las solicitudes de subvención, no debe ser utilizada sin el permiso explícito y por escrito del autor de la obra involucrada en dichos servicios.

7. Fraude científico[6]

Criterios:

7.1. El fraude en la publicación científica hace referencia a la presentación de datos o conclusiones falsas que no fueron generados a través de un proceso riguroso de investigación.
7.2. Existen los siguientes tipos de fraude en la publicación de resultados de investigación:
a) Fabricación de datos. Inventar datos y resultados de investigación para después comunicarlos.
b) Falsificación de datos. La manipulación de materiales de investigación, imágenes, datos, equipo o procesos.
La falsificación incluye la modificación u omisión de datos o resultados de tal forma que la investigación no se representa de manera precisa. Una persona podría falsificar datos para adecuarla al resultado final deseado de un estudio.

Recomendaciones:

7.3. Antes de enviar un artículo, lea cuidadosamente las políticas editoriales y de datos de la revista.
7.4. Nunca modifique, cambie u omita datos de forma intencional. Esto incluye materiales de investigación, procesos, equipos, tablas, citas y referencias bibliográficas.
7.5. Tanto la fabricación como la falsificación de datos son formas de conducta incorrecta graves porque ambas resultan en publicaciones científicas que no reflejan con precisión la verdad observada.
7.6. El autor debe hacer una gestión adecuada de los datos que soportan la investigación, teniendo especial cuidado en la recopilación, producción, conservación, análisis y comunicación de los datos.
7.7. Mantenga registros minuciosos de los datos en bruto, los cuales deberán ser accesibles en caso de que un editor los solicite incluso después de publicado el artículo.

8. Plagio[7]

Criterios:

8.1. El plagio es una de las formas más comunes de conducta incorrecta en las publicaciones, sucede cuando uno de los autores hace pasar como propio el trabajo de otros sin permiso, mención o reconocimiento. El plagio se presenta bajo formas diferentes, desde la copia literal hasta el parafraseado del trabajo de otra persona, incluyendo: datos, ideas, conceptos, palabras y frases.
8.2. El plagio tiene diferentes niveles de gravedad, como por ejemplo:
a) Qué cantidad del trabajo de otra persona se tomó (varias líneas, párrafos, páginas, todo el artículo)
b) Qué es lo que se copió (resultados, métodos o sección de introducción).
8.3. El plagio en todas sus formas constituye una conducta no ética editorial y es inaceptable.
8.4. La copia literal solo es aceptable si indica la fuente e incluye el texto copiado entre comillas.

Recomendaciones:

8.5. Recuerde siempre que es esencial reconocer el trabajo de otros (incluidos el trabajo de su asesor o su propio trabajo previo) como parte del proceso.
8.6. No reproduzca un trabajo palabra por palabra, en su totalidad o en parte, sin permiso y mención de la fuente original.
8.7. Mantenga un registro de las fuentes que utiliza al investigar y dónde las utilizó en su artículo.
8.8. Asegúrese de reconocer completamente y citar de forma adecuada la fuente original en su artículo.
8.9. Incluso cuando haga referencia a la fuente, evite utilizar el trabajo de otras personas palabra por palabra salvo que lo haga entre comillas.
8.10. El parafraseado solo es aceptable si indica correctamente la fuente y se asegura de no cambiar el significado de la intención de la fuente.
8.11. Incluya entre comillas y cite todo el contenido que haya tomado de una fuente publicada anteriormente, incluso si lo está diciendo con sus propias palabras.

9. Fragmentación[8]

Criterios:

9.1. La fragmentación consiste en dividir o segmentar un estudio grande en dos o más publicaciones.
9.2. Como norma general, con tal de que los "fragmentos" de un estudio dividido compartan las mismas hipótesis, población y métodos, no se considera una práctica aceptable.
9.3. El mismo "fragmento" no se debe publicar nunca másde una vez. El motivo es que la fragmentación puede dar lugar a una distorsión de la literatura haciendo creer equivocadamente a los lectores que los datos presentados en cada fragmento (es decir, artículo de revista) se derivan de una muestra de sujetos diferente. Esto no solamente sesga la "base de datos científica", sino que crea repetición que hace perder el tiempo de los editores y revisores, que deben ocuparse de cada trabajo por separado. Además, se infla injustamente el número de referencias donde aparece citado el autor.

Recomendaciones:

9.4. Evite dividir inapropiadamente los datos de un solo estudio en dos o más trabajos.
9.5. Cuando presente un trabajo, sea transparente. Envíe copias de los manuscritos estrechamente relacionados al manuscrito en

cuestión. Esto incluye manuscritos publicados, enviados recientemente o ya aceptados.

10. Consentimiento informado

Criterios:

10.1. Los estudios sobre pacientes o voluntarios requieren la aprobación de un comité de ética.

10.2. El consentimiento informado debe estar debidamente documentado.

10.3. Los permisos y las liberaciones deben ser obtenidos, cuando un autor desea incluir detalles de caso u otra información personal o imágenes de los pacientes y cualquier otra persona.

10.4. Especial cuidado debe tenerse con la obtención del consentimiento respecto a los niños (en particular cuando un niño tiene necesidades especiales o problemas de aprendizaje), donde aparece la cabeza o la cara de una persona, o cuando se hace referencia al nombre de un individuo u otros datos personales.

11. Corrección de artículos publicados[9]

Criterio:

Cuando un autor descubre un error o inexactitud significativa en el trabajo publicado, es obligación del autor notificar de inmediato a la revista y cooperar en el proceso de corrección.

Referencias

Black, William, Rodolfo Russo, y David Turton. «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes». Physics Letters B 694, n.º 3 (noviembre de 2010): 246-51.

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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.

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