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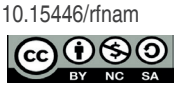

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EVALUADORES

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

Siete décadas de Ecología y Ciencias Forestales en la FCA

La primera referencia documentada de que se tenga conocimiento en Colombia sobre la enseñanza de la Ecología, como asignatura regular en un programa universitario de pregrado, data de 1954. Esto no desconoce que, desde finales del siglo XVIII Mutis con su “Cátedra de Matemáticas”, y luego en los siglos XIX y XX varios naturalistas, botánicos y geógrafos nacionales y extranjeros, hayan ofrecido cursos y palestras que gravitan en el entorno de lo que hoy se conoce como la “Ciencia de la Ecología”.

A mediados de 1954 arribó a Medellín el profesor de la Universidad del Estado de Michigan (MSU) Forrest Dean Freeland, quien recientemente había presentado su tesis doctoral sobre un tema de Hidrología Forestal en la propia MSU; él hacía parte de un programa de cooperación entre esa universidad y la Universidad Nacional de Colombia que se desarrolló para las sedes Medellín y Palmira en las áreas de agricultura y bosques, derivado del Punto Cuatro del programa de gobierno del presidente de los Estados Unidos Harry S. Truman, que trataba de la asistencia a países poco desarrollados luego de la Gran Guerra. Freeland llegó al recién creado Instituto Forestal de la Facultad Nacional de Agronomía en Medellín, para apoyar académicamente el programa de Ingeniería Forestal, el primero creado en el País, y que adelantaba en ese entonces la promoción de la primera cohorte de Ingenieros Forestales que habrían de graduarse en la Universidad.

Ese hito histórico relevante en realidad tenía otros antecedentes importantes en la Facultad. En el primer programa de estudios aprobado para la carrera de Ingeniería Agronómica de 1916 se incluyó la asignatura “Selvicultura”, a fin de formar a estos profesionales en los principios de manejo de bosques, su aprovechamiento y cuidado, y sus relaciones con la conservación del agua, del suelo y de la fauna silvestre.

Si bien los primeros egresados de la década de los años veinte del siglo pasado hacia monografías y trabajos finales en temas referidos fundamentalmente a los cultivos agronómicos y la producción animal, uno de ellos, el Ingeniero Agrónomo Federico Drews, presentó en 1925 su trabajo titulado “Bosques y su explotación".

Hay pues una dilatada historia de interés de la Facultad por los temas forestales y ecológicos, que se materializaron con la creación del Instituto Forestal y de la carrera de Ingeniería Forestal en 1951, la primera como unidad pionera en el estudio de las ciencias forestales en Colombia, y de la Ecología como ya se mencionó a partir de la cátedra de Freeland.

En el año de 2021 fue entonces la notable celebración de la efeméride de los 70 Años del Departamento de Ciencias Forestales y de la carrera de Ingeniería Forestal; tantas décadas orientando esfuerzos académicos, investigación y formación de pregrado y de posgrado en ciencias forestales, llenan de orgullo a esta Facultad y a todos sus profesores, estudiantes y egresados. La ocasión fue también el motivo para reiterar que la Facultad y su Departamento de Ciencias Forestales continuará en su propósito de seguir aportando al desarrollo de la cultura forestal y ecológica del país.

GUILLERMO VÁSQUEZ VELÁSQUEZ
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Bioclimatic performance of wet coffee processing facilities: conditions for workers and coffee

UNIVERSIDAD NACIONAL DE COLOMBIA

Desempeño bioclimático de instalaciones de procesamiento de café húmedo: condiciones para los trabajadores y el café

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Lina Marcela Guerra García[1], Robinson Osorio Hernández[1]*, Jairo Alexander Osorio Saráz[1], Joyce Correna Carlo[2] and Flavio Alves Damasceno[3]

Keywords:
Computational simulation
Hygrothermal behavior
Post-harvest coffee
Thermal comfort
WBGT index.

ABSTRACT

This study aimed to evaluate the bioclimatic performance of three wet coffee processing facilities in Colombia, focused on the conditions for workers and coffee parchment, through computer simulation. In addition to temperature and relative humidity, the Wet-Bulb Globe Temperature index (WBGT) was simulated during the highest coffee production month. The proposed simulation model was able to predict hygrothermal behavior within the three coffee processing facilities. Case 3 presented the warmest environment, and case 2 the most humid environment concerning the appropriate conditions for the coffee and the worker. The WBGT index limit was exceeded in case 3. Since this type of facility emits large amounts of heat and steam, constructive modifications are suggested to improve the environmental conditions of workers and coffee. Mainly, the physical separation of the heat exchangers is recommended, which ideally should be outside the post-harvest facility. The steam produced in the drying process should be quickly evacuated with ventilation strategies. Additionally, the use of strategies that reduce the energy gain from solar radiation is suggested.

Palabras clave:
Simulación computacional
Ambiente higrotérmico
Poscosecha de café
Confort térmico
Índice WBGT

RESUMEN

Este estudio tuvo como objetivo evaluar el desempeño bioclimático de tres instalaciones de procesamiento húmedo de café en Colombia, enfocado en las condiciones para los trabajadores y el café pergamino, a través de simulación computacional. Además de la temperatura y la humedad relativa, se simuló el índice de temperatura del globo negro y bulbo húmedo (WBGT) durante el mes de mayor producción de café. El modelo de simulación propuesto pudo predecir el comportamiento higrotérmico dentro de las tres instalaciones de procesamiento de café. El caso 3 presentó el ambiente más cálido, y el caso 2 el ambiente más húmedo con respecto a las condiciones adecuadas para el café y el trabajador. En el caso 3 se superó el límite del índice WBGT. Dado que este tipo de instalaciones emite grandes cantidades de calor y vapor, se sugieren modificaciones constructivas para mejorar las condiciones ambientales de los trabajadores y el café. Principalmente, se recomienda la separación física de los intercambiadores de calor, que idealmente deberían estar fuera de la instalación de poscosecha. El vapor producido en el secado debe evacuarse rápidamente con estrategias de ventilación. Además, se sugiere el uso de estrategias que reduzcan la ganancia de energía de radiación solar.

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In Colombia, coffee is mainly processed wet, through sub-processes that include pulping, fermentation, washing, and drying of the beans. The bioclimatic conditions of the facilities where these processes take place can be decisive for both the comfort of the workers (Guerra-García *et al*., 2019) and the quality preservation of dry parchment coffee. The coffee is often stored there for a long time (sometimes exceeding two weeks), where a large amount of heat and steam is generated by the machines during mechanical drying (Osorio *et al*., 2018; Osorio *et al*., 2020).

On the one hand, according to Todd and Valleron (2015), the heat generated by humans increases with increasing physical activity. Roscani *et al*. (2017) and Gutiérrez *et al*. (2018) stated that the combination of this situation with a stressful thermal environment can result in considerable occupational hazards. Workers in the wet coffee process develop physically demanding activities, in a hot and humid environment, which can generate heat stress, weakness in the general state of health, alterations in psychosensory reactions, and decreased productivity (Roscani *et al*. 2017; ISTAS-C, 2017; Gutiérrez *et al*. 2018).

For the evaluation of workers' hygrothermal environment, the Ministry of Labor and Social Security of Colombia proposes that the appropriate temperature range should be between 14 and 25°C, and the relative humidity between 30 and 70%. For a more complete comfort analysis, the use of comfort indices is required.

To evaluate human performance in work under conditions of exposure to heat, different standards have defined the use of the Wet Bulb Index and Globe Thermometer (WBGT). Among them, Resolution 2400 of 1979 establishes some provisions on housing, hygiene, and safety in the workplace in Colombia (MTSS, 1979), the international standard ISO 7243, (1982) estimates the heat stress of workers, as well as the Brazilian regulatory standard - NR 15, attachment n. 3 (ABNT-NBR, 2011). These last two standards also establish the lines for calculating the WBGT index, in addition to proposing its limits.

The WBGT index is an indicator of thermal comfort that considers air temperature, metabolism, radiant heat, air velocity, and relative humidity as the main drivers of thermal overload (MTSS, 1979; ISO 7243, 1982; ABNT-NBR, 2011; Roscani *et al*. 2017). Guerra-García *et al*. (2019) used this index for comfort analysis in coffee post-harvest facilities in Colombia, obtaining satisfactory results.

On the other hand, the quality of dry parchment coffee can be compromised during storage due to the hygrothermal conditions of the environment. According to Puerta (2016), The quality of dry parchment coffee stored in Colombia, with humidity between 10 and 12% in the bean, can be preserved for a maximum of six months, with an air temperature below 20 °C and relative humidity between 60 and 70%. Different environmental conditions ultimately affect the taste and quality of the coffee drink.

Studies on the bioclimatic performance of facilities for wet coffee processing are few. The available literature suggests that these buildings present unsuitable storage environments that can affect the quality of the coffee (Osorio *et al*., 2020). Additionally, there is very little research that evaluates the thermal comfort of workers within post-harvest facilities (Guerra-García *et al*., 2019). According to Herrera *et al*. (2015), this is a widespread problem in the food industry, mainly in Latin America and the Caribbean.

Computational simulation has become a useful tool for the design and bioclimatic evaluation of buildings since it allows the analysis of mass and energy transfer phenomena, internal loads, occupancy patterns, transitory analysis, among others (Al-Saadi and Al-Jabri, 2020). However, in the evaluation of the built environment, the computational simulation models must be validated with experimental data to obtain reliable results.

One of the most internationally recognized bioclimatic and energy simulation software for buildings is EnergyPlus™. This open-source software, developed by the US Department of Energy (DoE), is capable of simulating long periods of time with the help of local weather files. The EnergyPlus™ program is a collection of many program modules that work together to calculate the energy required for air conditioning of buildings using 3D geometries, climate files, internal loads (machines and metabolism), usage patterns, thermal properties of materials, among others (DoE, 2019).

The EnergyPlus™ software allows generating computational models to evaluate the bioclimatic performance of coffee

post-harvest facilities (Osorio *et al*., 2018), seeking solutions that benefit both the conservation of parchment coffee and the thermal comfort of the worker (Guerra-Gracía *et al*., 2019). This study aimed to evaluate the bioclimatic performance of three wet coffee processing plants in Colombia, focused on the comfort conditions of the workers and the environmental conditions for the conservation of parchment coffee.

MATERIALS AND METHODS

Three case studies corresponding to three wet coffee processing facilities were compared, which from now on will be called case 1, 2, and 3. Except for their architecture, the three cases have similar geographic location conditions (municipalities of the north region of Antioquia - Colombia), mechanical drying, annual production of 30 t of dry parchment coffee, and peak production in November. Table 1 summarizes the description of the location and architectural details of the three case studies.

Data of air temperature and relative humidity inside the three study cases were collected employing an Extech Instruments datalogger of the RHT20 brand, using a temperature measurement interval between -40 and 70 °C with a resolution of 0.1 °C, as well as a range between 0 and 100% relative humidity, with a resolution of 0.1. The collections were carried out every 5 min during three continuous days in each case, in November 2016, by fixing the equipment in the geometric center of each installation.

Table 1. Description of location and architectural details of the case studies.

Item	Case 1	Case 2	Case 3
Location: coordinates, altitude and municipality.	6°24'47,64"N, 75°20'47,08"W, 1508 masl, Barbosa.	6°21'42,16"N, 75°34'44,83"W, 1698 masl, Bello.	6°29'22,79"N, 75°00'41,86"W, 1435 masl, San Roque.
Architectural configuration	Flat construction. Chimney over the drying area, cherry coffee receipt dry hopper on the slab.	Two-level flat construction. Wet process and coffee drying process on the first level, cherry coffee receipt dry hopper, and housing on the second level. Annex buildings.	Staggered construction. Wet and dry process on different levels, cherry coffee receipt dry hopper on the fourth level.
Buildings dimensions	Width: 6.0 m Length: 11.0 m Height: 3.50 m	Width: 9.10 m Length: 10.05 m Height: 3.60 and 3.80 m	Width: 5.10 m Length: 14.50 m Height: 2.40, 1.90 and 2.45 m
Wall materials	Exposed perforated brick	Brick plastered up to a height of 2.0 m, exposed to the roof.	Brick wall, ceramic cladding.
Roof materials	Slab lightened with bricks	Slab lightened with bricks in the first part, and fiber cement tile in the second part.	Slab lightened with bricks on the upper level, and aluminum tile on the lower levels.
Floor materials	Exposed concrete	Exposed concrete	Ceramic coated concrete.
Openings	Openwork brick to finish all the walls at the top.	Three doors and six openings with security bars.	Grille door in the first and last levels, window in the first level and openings with security grilles between slopes.

The computational simulation analysis was carried out in the EnergyPlus™ program, based on the weather file of the city of Medellín, due to its availability, proximity (linear distance from Medellín to Bello 12 km, Barbosa 34 km, and San Roque 65km), and a similar climate to the location zones of the facilities under study (DesignBuilder, 2017). For the simulation model, a 3D geometry of each case study was elaborated in the Sketchup® software. With the help of the OpenStudio plugin, thermal zones were created. Finally, files with an idf extension were generated for each case (Figure 1).

The thermal properties of construction materials such as density, thermal conductivity, and specific heat were obtained from the ABNT-NBR 15220-2 (2005). To simplify the 3D model in case 1, the areas with openwork brick were assumed as windows, adding sun protection to simulate interference with light (Osorio *et al.*, 2016). The mass of the material composed of brick and concrete, constituted by stairs, hydraulic classifier, and fermentation tanks was quantified and added to the immediate wall, to consider the effect of thermal inertia in the three cases.

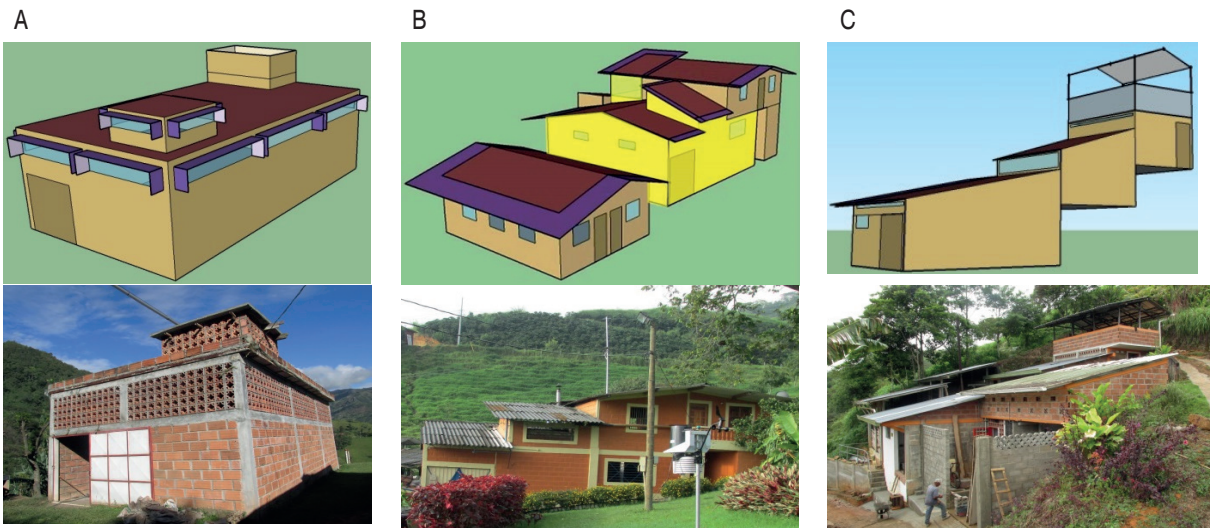

Figure 1. 3D geometries of the three case studies: A case 1, B case 2 (yellow area), and C case 3.

For the calculation of the energy balances (Equation 1), the internal loads were considered: powers of the coffee processing equipment (pulping machines and mechanical drying), and the luminaires (Table 2). Likewise, the heat generated from the human metabolism was considered as 440 kcal h^{-1} (512 W), corresponding to an intermittent work of lifting, pushing, or dragging weights (ABNT-NBR, 2011).

$$\frac{C_z dT_z}{dt} = \sum_{i=1}^{n} \dot{Q}_1 + \sum_{i=1}^{n} h_i A_i (T_{si} - T_z) + \sum_{i=1}^{n} \dot{m}_i C_p (T_{zi} - T_z) + \dot{m}_{inf} C_p (T_\infty - T_z) + \dot{Q}_{sys} \qquad (1)$$

where,

$\sum_{i=1}^{n} \dot{Q}_1$: Sum of internal convective loads (w);

$\sum_{i=1}^{n} h_i A_i (T_{si} - T_z)$: Convective heat transfers from area surfaces (w);

$\dot{m}_{inf} C_p (T_\infty - T_z)$: Heat transferred through the grooves (w);

$\sum_{i=1}^{n} \dot{m}_i C_p (T_{zi} - T_z)$: Heat transfer due to interzone air mixing (w);

$\dot{Q}_{sys}$: Air systems outlet (w);

$\frac{C_z dT_z}{dt}$:Stored energy (w);

C_z : $\rho_{air} C_p C_s$

ρ_{air} : Zone air density (kg m^{-3});

C_p: Specific heat of the air (J kg^{-1} $°K^{-1}$);

C_s: Sensible heat capacity multiplier (J kg^{-1} $°K^{-1}$).

Table 2. Power of machines for wet processing of coffee.

Equipment	Power	Usage patterns (h)
Pulping module	3357.0 W	11:00 - 12:00 and 17:00 - 18:00
Engine of fan of mechanical dryer	2238.0 W	6:00 - 19:00
Submersible pump *	746.0 W	16:00 - 16:30
Heat exchanger of mechanical dryer case 1	127570.6 W	6:00 - 18:00
Heat exchanger of mechanical dryer case 2	57381.8 W	6:00 - 18:00
Heat exchanger of mechanical dryer case 3	156053.3 W	6:00 - 18:00
Luminaires	10 W m^{-2}	17:00 - 20:00

* Only present in case 3

The simulation was carried out for the entire month of November in each case, from hour to hour, to determine the bioclimatic performance of the facilities during the peak period of coffee production, specifically for analyzing the conditions for the workers and parchment coffee. Table 3 shows the schedules used for the computational simulation in EnergyPlus™.

For the validation of the computational model, the experimental data (720 data) of temperature and relative humidity of the air was used to calculate the Normal Mean Square Error (NMSE) (ASTM, 2002) (Equation 2). NMSE values less than 0.25 are considered acceptable and good indicators of concordance. The agreement between the experimental values and the simulated values increases as the value is closer to zero.

Table 3. Schedules used for the computational simulation.

Hours	Activities
6:00:00	Fuel feed for drying coffee
8:00:00	Breakfast
9:00:00	Washing of parchment coffee
10:00:00	Hydraulic classification of coffee
11:00:00	Pulping
12:00:00	Lowering the coffee layer inside the three-layer dryer
13:00:00	Lunch
14:00:00	Fuel feed for drying coffee
15:00:00	Washing of parchment coffee
16:00:00	Hydraulic classification of coffee
17:00:00	Pulping
18:00:00	Lowering the coffee layer inside the three-layer dryer
19:00:00	Packing of parchment coffee

$$NMSE = \frac{1}{n}\sum_{i=1}^{n}\frac{(Y_{pi} - Y_{mi})^2}{Y_{pi}Y_{mi}} \quad (2)$$

where Y_{pi} is the predicted value, Y_{mi} is the measured value, and n is the number of data.

Equation 3 was used to analyze the WBGT index in the three case studies from the simulations. It is suitable for the evaluation of internal environments (without solar load), according to NR 15, annex n. 3 (ABNT-NBR, 2011).

$$WBGT=0.7T_W+0.3T_g \quad (3)$$

Where:
T_W: Natural wet bulb temperature, °C; and
T_g: Black globe temperature, °C.

Since EnergyPlus™ provides no output data for the WBGT index, it was calculated from other output data that can be obtained through the software: the black globe temperature (T_g) was cleared from the Radiant Mean Temperature (TRM) equation, proposed by Fanger (1972). In Equation 4, TRM, T_{db} and V_a are data provided by the software.

$$TRM = T_g + 1.9\sqrt{V_a}(T_g + T_{db}) \quad (4)$$

Where:
T_g: Black globe temperature, (°C);
T_{db}: Dry bulb temperature, (°C); and
V_a: Air speed, (m s^{-1}).

The Wet Bulb Temperature was obtained by Equation 5 (Stull, 2011), using data of T_{db} and relative humidity of the air, supplied by EnergyPlus™.

$$Tw = T_{db} \times \operatorname{atan}\left[0.151977(RH + 8.313659)^{\frac{1}{2}}\right] + \operatorname{atan}(T_{db} + RH) - \operatorname{atan}(RH - 1.676331) + 0.00391838(RH)^{\frac{3}{2}} \times \operatorname{atan}(0.023101RH) - 4.686035 \quad (5)$$

Where T_W: wet bulb temperature, (°C); T_{db}: dry bulb temperature and RH: relative air humidity (%).

The tolerance limits of workers to heat exposure based on the WBGT index (°C) were determined by considering the most repetitive work in the three study cases, that is, lifting, pushing, or dragging heavy loads, with a metabolic rate of 440 kcal h^{-1} (512 W), classified as heavy according to (ABNT-NBR, 2011). Based on these parameters, it was determined that the limit for the WBGT index of heavy activity, developed continuously during 1 h is 25 °C (ABNT-NBR, 2011).

The WBGT index and hygrothermal behavior of the three study cases and of these cases with the external environment according to the simulations were compared using hourly data of November 2016. For this comparison, box plots were made for the three cases.

Results were compared with the temperature of 20 °C and relative humidity of the air between 65 and 70% recommended by Puerta (2016) to preserve the quality of the stored dry parchment coffee.

RESULTS AND DISCUSSION

Table 4 shows the results of validation of the models for the dry bulb temperature and relative humidity of the air, in the three study cases. The value of the NMSE in the three cases is less than 0.25, that is, the models represent the real conditions (ASTM, 2002), as well as the agreement between the measured and simulated temperature data for the three cases is observed in Figure 2.

Table 4 . Comparison of temperature and relative humidity of the air from experimental and simulation data with NMSE.

Facilities for wet coffee processing		Temperature (°C)		Relative humidity (%)	
		Measured	Predicted	Measured	Predicted
Case 1	Average	21.6	22.1	74.1	73.7
	NMSE	0.02089		0.01455	
Case 2	Average	20.0	20.8	86.1	83.3
	NMSE	0.01776		0.02256	
Case 3	Average	25.7	23.5	79.6	79.2
	NMSE	0.03372		0.02670	

Therefore, it is possible to affirm that the proposed simulation models are capable of predicting the hygrothermal behavior within the three facilities. The models can facilitate decision-making to improve the behavior of environmental variables within this type of construction.

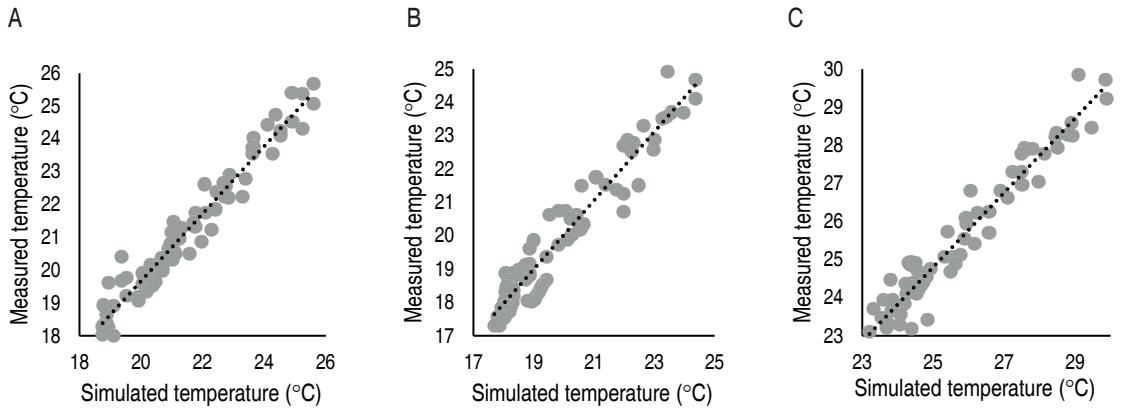

Figure 2. Comparison of measured data and simulated temperature data, A. case 1, B. case 2, C. case 3.

According to the simulations in EnergyPlus™, Figures 3A and 4A show the thermal behavior of the three cases and the external environment on an average day in November, and the general behavior in a box diagram, respectively. It is observed that the warmest environment occurs in case 3, and the coolest environment in case 2. Concerning workers, the temperature range must be between 14 and 25 °C (MTSS, 1979) and it is observed that the low limit is not exceeded. However, in the warmest hours of the day when there was greater solar radiation, case 3 exceeded the value of 30 °C, thus generating an environment of thermal stress at these hours. This effect can be corrected with passive strategies of natural ventilation.

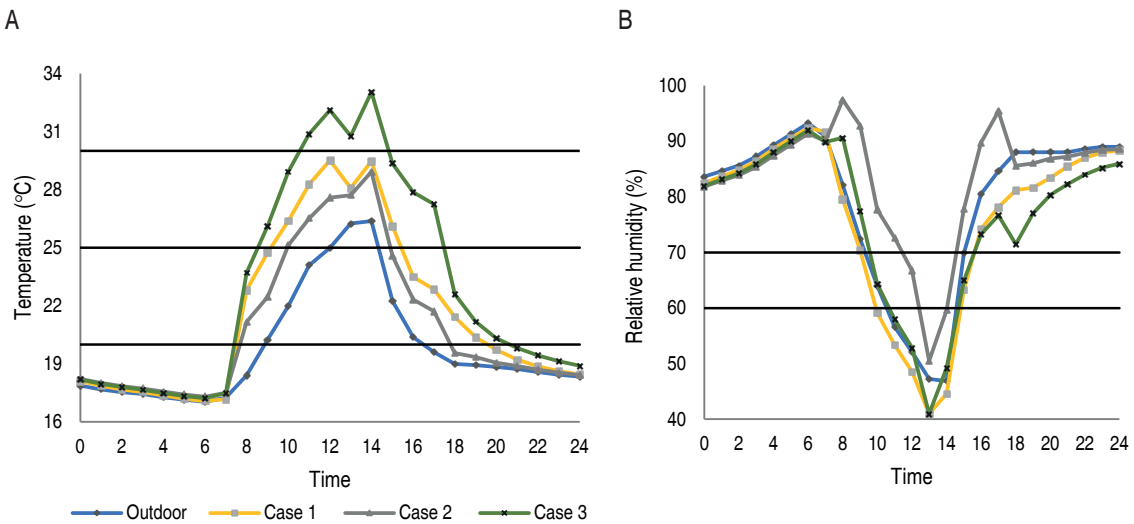

Figure 3. A. Average day temperature, B. Relative humidity of an average day of November for the three study cases.

For coffee, in the three study cases more than half of the time, the temperature was higher than the recommended limit to preserve the quality of the coffee (20 °C in storage). Similar to the behavior of the external environment, this behavior occurs during daylight hours (Figure 3A). Case 3 was the most critical, since more than 80% of the time the temperature was above 20 °C. In this case, the temperature above the desired limit can be mitigated with passive strategies that decrease heat transfer by solar radiation, for example, the use of light or reflective colors on the roof.

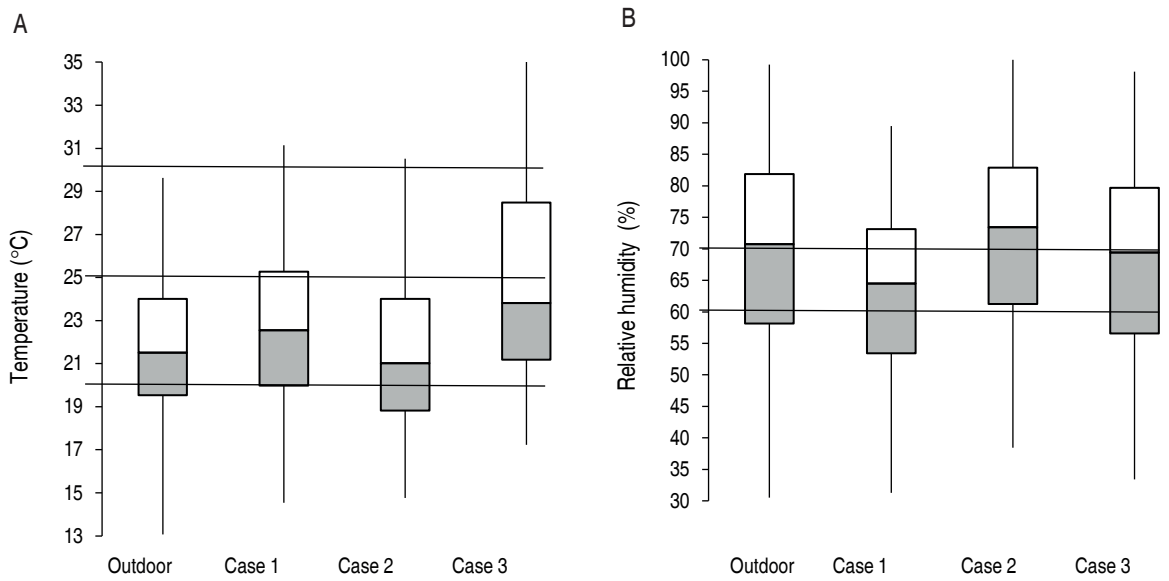

Figure 4. Box plot for the three case studies for November: A. temperature, B. relative humidity.

In case 1 and case 3, the heat exchangers of the coffee drying machines, which work with coffee husk and firewood, respectively (as the energy source for the drying process), had an impact on the increase in internal temperature, since their efficiency was 50%. The rest of the energy is emitted to the environment in the form of sensible heat. Additionally, in case 3 the aluminum cover (material with high thermal conductivity) and its low height contributed to higher temperatures during the hottest hours of the day.

In case 2, there was a lower temperature inside because the drying machine has no heat exchanger. The coffee is dried with the direct flame from propane gas, therefore, the process emits less heat to the environment (Oliveros *et al*., 2009). Additionally, the construction has a large volume and receives little solar radiation due to neighboring buildings. However, this is not enough to keep the values within the recommended limit to preserve the quality of dry parchment coffee.

Figures 3B and 4B show the relative humidity behavior of the three cases and the external environment on an average day in November, and the general behavior in the box plot according to the simulations, respectively. It was observed that case 2 and case 1 presented the most and least humid environments. In Figure 5, referred to workers, the relative humidity range should be between 30 and 70%. (MTSS, 1979), but it was observed that during the morning and at night this value was exceeded due to the steam emitted by the facility in the drying process and the reduction in the external temperature. Nevertheless, the relative humidity did not fall below 40%.

Figure 4B shows a wide range of relative humidity in the external environment, with values between 30% and close to 100%, due to the rains during the coffee harvest season in the area but also to some dry days with clear sky. Similar behavior was found in the case studies. Most of the data were outside the recommended range to preserve the quality of coffee (60 to 70%), and for the wellbeing of workers (30 to 70%). The most humid and critical environment was case 2.

Case 1 has a greater area of natural ventilation openings; thus the relative humidity was lower, followed by case 3, with 32% and 49% of data above 70% relative humidity, respectively. In case 2, the area of ventilation openings is not sufficient for its volume, making it difficult to evacuate the steam generated by the drying process. This caused that 60% of the data exceeded the value of 70% relative humidity. This case represents a great biological risk for the proliferation of fungi and bacteria in coffee.

The hygrothermal performance results indicate that none of the three facilities is suitable for the storage of dry parchment coffee all the time, since they require modifications through passive conditioning methods or the use of active conditioning systems as last resort. Regarding the comfort level of workers in coffee post-harvest facilities, the behavior of the WBGT index, calculated from the results of the simulations of the three case studies, can be observed in Figure 5.

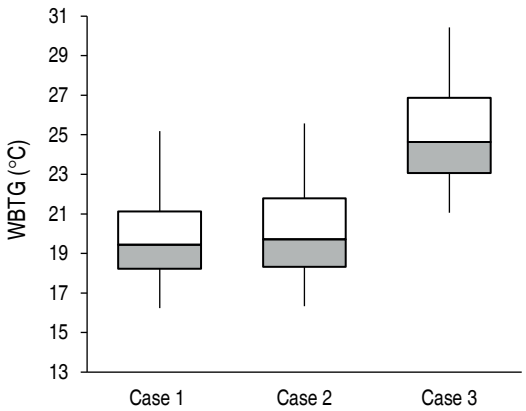

Figure 5. Box plot of WBGT index in the three case studies.

It was found that the WBGT index exceeded 45% of the time in case 3, 3.3% in case 2 and less than 1% in case 1. Data that exceeded the limit of the index appeared due to both the high temperature and the humidity of the air, mainly because of the combination of wet coffee and drying processes within the same facilities. This hinders the loss of heat by perspiration (latent heat) and causes thermal stress (Roscani *et al*. 2017; ISTAS-C, 2017; Gutiérrez *et al*. 2018).

It is possible to state that, with the predominance of values lower than the 25 °C limits in the three cases, the WBGT index does not represent a difficult problem, since it could be solved with the application of passive conditioning methods, mainly the physical separation of the spaces of the drying processes from those that generate heat and humidity in the environment. In other words, the heat exchangers of the drying machines should ideally be located externally to the post-harvest facility, and the steam produced in the drying must be quickly evacuated from the building, either with ventilation openings close to the steam outlet and favoring the chimney effect (Osorio *et al*., 2020) or with designs where the construction drying machine stands out.

CONCLUSIONS

The proposed computational model is capable of predicting hygrothermal behavior, within the three case studies of facilities for wet coffee processing. Case 1 presented the best hygrothermal and comfort conditions, due to its design with the largest natural ventilation area and the largest volume of the three cases. Case 2 presented the most humid environment, and case 3 the warmest environment, which increases the biological risk for coffee storage. Regarding the WBGT index, case 3 presented the most unfavorable condition.

To improve the environmental conditions for the workers and the coffee, constructive modifications are suggested. First of all, the heat exchangers of the dryers should ideally be located outside the post-harvest facility. Secondly, the steam produced in the drying should be quickly evacuated from the building, either with ventilation openings close to the steam outlet that favor the chimney effect or with designs where the construction dryer protrudes from the roof. Additionally, passive air conditioning strategies should be implemented to reduce the gain of solar radiation, such as painting the ceiling in light colors or with reflective colors or materials.

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Epidemiological study of *Colletotrichum* spp. associated with *Enterolobium cyclocarpum* (Jacq.) Griseb. and *Platymiscium pinnatum* (Jacq.) Dugand in the Colombian Caribbean Region

Estudio epidemiológico de *Colletotrichum* spp. asociado a *Enterolobium cyclocarpum* (Jacq.) Griseb. y *Platymiscium pinnatum* (Jacq.) Dugand en la region caribe colombiana

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Keywords:
Development curves
Disease development rate
Foliar diseases
Incidence
Severity

ABSTRACT

Epidemiological analyzes of foliar diseases associated with *Colletotrichum* spp. in *Enterolobium cyclocarpum* and *Platymiscium pinnatum* were performed under field conditions and without any type of intervention. At the Universidad del Magdalena (Santa Marta, Colombia), four trees for each species and four equidistant monitoring sites per tree were established. The incidence and severity were recorded for 33 weeks (March to November 2016), including two follow-up periods: dry and rainy season. Disease development curves were elaborated. Moreover, the development rate (*r*) and the area under the disease progress curve (*AUDPC*) were calculated for each follow-up period. The effect of the meteorological variables was statistically analyzed by correlation and multiple regression. In *E. cyclocarpum*, the highest incidence and severity were recorded between September and November with 100 and 19.6%, respectively, showing a positive correlation with relative humidity and negative with average temperature, solar radiation and wind speed. In *P. pinnatum*, the maximum values of incidence and severity were observed between March and April with 68.9 and 1.3%, respectively. However, correlation analyzes did not support their relationship with the environmental factors. The *r* values during the dry months were 0.136 and 0.107 units week^{-1} and the *AUDPCs* were calculated at 51 and 4 units week^{-1} for *E. cyclocarpum* and *P. pinnatum*, respectively. In the rainy months, the *r* values were 0.187 and 0.016 units week^{-1} and the *AUDPCs* were 186 and 2 units week^{-1}, respectively. In conclusion, the development of the disease varies according to the forest species, time of year and some meteorological variables.

Palabras clave:
Curvas de desarrollo
Tasa de desarrollo de la enfermedad
Enfermedades foliares
Incidencia
Severidad

RESUMEN

Se realizó un análisis epidemiológico de enfermedades foliares asociadas a *Colletotrichum* spp. en *Enterolobium cyclocarpum* y *Platymiscium pinnatum* bajo condiciones de campo y sin ningún tipo de intervención. En el campus de la Universidad del Magdalena (Santa Marta, Colombia), se seleccionaron cuatro árboles de cada especie forestal y cuatro sitios equidistantes de seguimiento por árbol. La incidencia y severidad fueron registradas durante 33 semanas (de marzo a noviembre, 2016), incluyendo dos periodos de seguimiento: seco y lluvioso. Se elaboraron curvas de desarrollo de la enfermedad. Además, la tasa de desarrollo (*r*) y el área bajo la curva del progreso de la enfermedad (*AUDPC*) fueron calculadas para cada periodo de seguimiento. El efecto de las variables meteorológicas fue estadísticamente analizado mediante correlación y regresión múltiple. En *E. cyclocarpum*, el valor más alto de incidencia y severidad fueron registrados entre septiembre y noviembre con 100 y 19,6%, respectivamente, mostrando una correlación positiva con la humedad relativa y negativa con la temperatura promedio, la radiación solar y la velocidad del viento. En *P. pinnatum*, los valores máximos de incidencia y severidad fueron observados entre marzo y abril con 68,9 y 1,3%, respectivamente. Sin embargo, los análisis de correlación no confirmaron su relación con los factores ambientales. Los valores de *r* durante los meses secos fueron de 0,136 y 0,107 unidades semana^{-1} y las *AUDPCs* fueron calculadas en 51 y 4 unidades semana^{-1} para *E. cyclocarpum* y *P. pinnatum*, respectivamente. En los meses lluviosos, los valores *r* fueron de 0,187 y 0,016 unidades semana^{-1} y las *AUDPCs* fueron 186 y 2 unidades semana^{-1}, respectivamente. Se concluyó que el desarrollo de la enfermedad varía según la especie forestal, la época del año y algunas variables meteorológicas.

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Enterolobium cyclocarpum (Jacq.) Griseb. and *Platymiscium pinnatum* (Jacq.) Dugand (Fabaceae family) are common trees of the Colombian Caribbean region, with a high presence in the city of Santa Marta (department of Magdalena). *E. cyclocarpum* (subfamily: Mimosoideae) is distributed throughout the dry forests of Central America and northern South America (Rocha *et al*., 2018). This fabaceous is a species widely known for its uses in different human activities and, in forestry systems, its wood is used for construction, production of household utensils, furniture, musical instruments, among others (Pacheco *et al*., 2012). Its wide canopy provides shade appreciated in silvopastoral systems, as well as in urban centers, where it is planted as an ornamental tree along with parks and roads (Cordero and Boshier, 2003). Additionally, its fruits and seeds are suitable for human and animal consumption, making it possible to produce flours (Cordero and Boshier, 2003). On the other hand, extracts from different parts of the tree have been used to alleviate various ailments in humans and animals (Pacheco *et al*., 2012).

Platymiscium pinnatum (subfamily: Faboideae) is a neotropical tree distributed from Mexico to Brazil, preferably occupying dry habitats; however, its growth is common in humid ecosystems (Saslis-Lagoudakis *et al*., 2008). *P. pinnatum* is highly valued in forestry for its wood, which is used for multiple purposes, such as construction, fine wood carvings, furniture making, and musical instruments (Cordero and Boshier, 2003). This species has been described as a nitrogen fixer, being ideal for agroforestry systems and enrichment of degraded forests (Cordero and Boshier, 2003). In Central America, for example, *P. pinnatum* has been associated with organic and conventional agroforestry systems of shade coffee (Haggar *et al*., 2015). On the other hand, the use of leaf infusions has been reported for the treatment of infectious diseases in the skin and eyes (Pabón *et al*., 2017).

In a survey of pathogens in the urban trees of Santa Marta, Colombia, *Colletotrichum* spp. were associated with foliar diseases in *E. cyclocarpum* and *P. pinnatum* (Cantillo, 2014). The symptoms associated with *Colletotrichum* in *E. cyclocarpum* consist of whitish rounded spots at the leaflet base on the leaf underside, while in *P. pinnatum*, the symptom corresponds to a light or dark brown anthracnose surrounded by a chlorotic halo (Cantillo, 2014; Restrepo-Leal and Rada-González, 2017). Furthermore, in previous tests, the fungal pathogenicity was verified in both developing and mature foliar tissues of these forest species with the expression of characteristic symptoms in each plant (Restrepo-Leal and Rada-González, 2017). Due to these symptoms, the name "foliar Anthracnose" was defined to refer to this disorder or pathology.

Colletotrichum is one of the most important phytopathogenic fungi in the world because it affects a large number of plant species, causing huge economical losses (Dean *et al*., 2012). Moreover, this pathogen has a regular occurrence in different forest species, where it mainly has a causal relationship with foliar diseases that are expressed as spots, blights or anthracnose (Arguedas-Gamboa and Cots-Ibiza, 2012). In general, *Colletotrichum* spp. cause the disease called Anthracnose and, in aerial organs, these fungi cause sunken necrotic lesions, subcircular or angular, where the acervuli are formed (Dean *et al*., 2012; Arguedas-Gamboa and Cots-Ibiza, 2012).

To develop efficient control strategies, it is necessary: *i*) to carry out epidemiological studies of the disease that allow understanding its behavior, including aspects related to the infective period (Miles *et al*., 2013), and *ii*) to know the effect of the environmental variables on the disease development (Moral *et al*., 2012). In this regard, some research indicate that *Colletotrichum* species can be inactive during dry periods and change to infective when temperatures oscillate between 25 and 30 °C, and the relative humidity is greater than 80% (Miles *et al*., 2013; Lima *et al*., 2015). The severity of the disease is conditioned by the intensity and duration of precipitation, duration of humidity on the leaf surface, luminosity, among others (Huertas-Palacios *et al*., 2009; Moral *et al*., 2012).

Plant health studies in forest species have been limited to describing the pathogens associated with the crops; similarly, the phytosanitary regulatory bodies in each country have focused on keeping the phytosanitary status updated and, with some exceptions, on designing dispersal models or indicating the distribution of pests that threat forest production (Cordero and Boshier, 2003; Arguedas-Gamboa and Cots-Ibiza, 2012). In Colombia and Latin America, there are few investigations related to the epidemiology of pathologies in forest species, and

Anthracnose caused by *Colletotrichum* species is not an exception.

Information on the development of *Colletotrichum* spp. in *E. cyclocarpum* and *P. pinnatum* is non-existent. Additionally, in Santa Marta and the Caribbean region, there are few studies on forest health, which makes it difficult to develop phytosanitary management strategies for trees. The previous situation has motivated the beginning of an investigative process on the interaction *Colletotrichum* spp. – forest species, raising the hypothesis that the epidemiological behavior of foliar anthracnose associated with *Colletotrichum* spp. varies between forest tree species. This study aimed to analyze the behavior of "foliar Anthracnose" associated with *Colletotrichum* spp. in *E. cyclocarpum* and *P. pinnatum* under field conditions.

MATERIALS AND METHODS

Area of study

The research was carried out in trees at the Universidad del Magdalena Campus, located in the city of Santa Marta (Colombia), in an area between 11°13'43" and 11°13'22" North latitude, and 74°11'00" and 74°11'16" West longitude, at an altitude of approximately 20 m. Santa Marta city presents an average temperature of 28.3 °C, average relative humidity of 76% and an average annual rainfall of 545 mm (IDEAM, 2014).

Four individuals of *E. cyclocarpum* and *P. pinnatum* were selected from a population of 97 and 40 trees, respectively. In each tree, four monitoring sites were marked, corresponding to the four cardinal points. Every monitoring site corresponded to five leaves located in the terminal part of a branch and positioned in the under-canopy layer.

Epidemiological variables

The epidemiological parameters were evaluated during 33 weeks, from March 26, 2016, to November 6, 2016, obtaining 29 measurements for each monitoring site. The number of leaflets with symptoms and the number of total leaflets were recorded; in this way, the incidence of *Colletotrichum* spp. at each monitored time was calculated. The severity of the disease was estimated according to scale diagrams designed for each forest species (Figure 1), following the severity scale described by Páez *et al.* (2003), with modifications. This scale involved six levels of affectation, as indicated in Table 1. Additionally, defoliation was determined for each monitoring site, based on the differences in the total records of leaves from one measurement with respect to the previous one. Based on weekly data, disease development curves were constructed for each forest species and Spearman correlations were made between these variables (incidence, severity and defoliation).

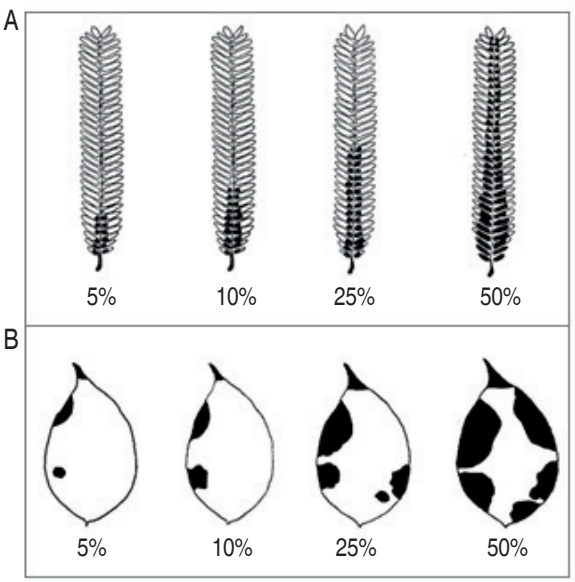

Figure 1. Scale diagrams to measure the severity of foliar Anthracnose (*Colletotrichum* spp.). A. Leaflets of *E. cyclocarpum*. B. Leaflets of *P. pinnatum*.

The disease development rate (*r*) was determined, according to Equation (1) proposed by Van der Plank (1963):

$$r = \frac{1}{\Delta_t}\left(\ln\frac{x_f}{1-x_f} - \ln\frac{x_i}{1-x_i} \right) \qquad (1)$$

where *r* is the rate of disease development, Δt is the time difference, x_f is the final value of the disease and x_i the initial value of the disease.

Table 1. Scale to quantify the anthracnose severity (*Colletotrichum* spp.).

Rank	%Affectation	Severity classification
0	0	Healthy
1	Up to 5	Very slight
2	6-10	Slight
3	11-25	Mild
4	26-50	Strong
5	>50	Very strong

Likewise, the area under the disease progress curve (*AUDPC*) was calculated, according to Equation (2) indicated by López-Vásquez *et al.* (2013):

$$AUDPC = \sum_{i}^{n-1}\left(\frac{y_i + y_{i-1}}{2} \right)(t_i - t_{i-1}) \qquad (2)$$

where *AUDPC* is the area under the disease progress curve, y_i is the final severity, y_{i-1} is the initial severity, t_i is the final time and t_{i-1} the initial time.

Both the development rate and the *AUDPC* were determined based on the severity values recorded for two follow-up periods: the first one, considered dry or with less rainfall, corresponding to March to June (first semester of the year); and the second one, considered rainy or with higher rainfall, between July and November (Table 2).

Effect of meteorological variables on the disease development

In order to know the effect of the meteorological variables on the behavior of foliar Anthracnose, a Spearman correlation analysis and a multiple regression analysis by ordinary least squares were performed between both incidence and severity with rainfall (mm), relative humidity (%), average temperature (°C), solar radiation (W m^{-2}) and wind speed (m s^{-1}). The meteorological data were provided by the Institute of Hydrology, Meteorology and Environmental Studies (IDEAM, 2014), according to the readings of the Meteorological Station of the Universidad del Magdalena. The measurements were analyzed weekly, averaging the values of each meteorological variable, except for rainfall, which was calculated cumulatively. All statistical analyzes were performed in Statgraphics® Centurion XVI program.

During the 33 weeks of field follow-up, the total rainfall was 402.2 mm. In the dry period (March-June) the accumulated rainfall was 82.7 mm, while in the rainy period (July-November), it was 319.5 mm, being October the rainiest month with 17.8 mm (Table 2).

From March to June, the highest values of temperature (average of 30 °C), solar radiation (average of 5961.0 W m^{-2}), and wind speed (average of 4.21 m s^{-1}) were recorded, while relative humidity (average of 68.1%) presented the lowest values during this follow-up period. In the second period, average temperature, solar radiation, and wind speed were 29 °C, 5298.0 W m^{-2} and 3.02 m s^{-1}, respectively. The average relative humidity was higher than the first semester with a value of 72.1% (Table 2).

This meteorological behavior is typical of the Colombian Caribbean region, where there is a unimodal rain distribution, with a short rainy period accentuated in the second semester of the year, and a long dry period that covers almost the entire first semester and some of the second. The temperature and relative humidity present fluctuating values throughout the year, with a slight increase in temperature in the first two months of the year and an increase of humidity in September to November, due to the effect of the rains. Wind speed is generally higher between December to March (IDEAM, 2014).

RESULTS AND DISCUSSION

Incidence and severity of foliar anthracnose and their relationship with tree defoliation

The mean of incidence and severity, for both follow-up periods (*i.e.* dry and rainy), are shown in Table 3. *E. cyclocarpum* presented higher values of the disease

Table 2. Meteorological data from 26-03-2016 to 06-11-2016 (33 weeks) at the Universidad del Magdalena, Santa Marta, Colombia. Rain: Rainfall. R.H: Relative humidity. Temp: Average temperature. Rad: Solar radiation. Wind: Wind speed.

Month	Day	Rain (mm)	R.H (%)	Temp (°C)	Rad (W m^{-2})	Wind (m s^{-1})
March	86	0.4	64.3	30.3	(no data)	(no data)
	93	0.0	65.3	29.7	6446.1	7.44
April	100	0.0	59.8	29.8	6962.2	5.35
	107	0.6	61.3	30.0	6759.7	4.12
	114	10.6	70.7	30.1	5586.1	5.56
	121	0.0	73.0	29.9	5574.0	2.02
May	128	5.4	73.0	29.9	5291.9	3.22
	135	13.8	69.1	30.3	5757.8	3.37
	143	0.0	64.3	31.8	6789.6	5.23
	150	0.8	70.9	29.9	5800.9	3.13
	156	7.6	75.1	29.5	5289.9	4.44
June	163	7.6	72.4	30.0	5630.4	3.58
	170	5.6	67.4	29.7	6032.7	4.66
	177	30.3	71.1	29.1	5510.4	2.97
	184	0.0	64.3	30.2	6022.3	3.88
Average*		**82.7**	**68.1**	**30.0**	**5961.0**	**4.21**
July	191	0.0	63.0	29.9	6322.6	4.66
	198	2.2	65.7	29.5	5175.2	3.50
	205	26.6	70.1	28.7	5665.4	3.57
	212	0.6	62.6	29.9	5298.1	4.52
August	219	5.9	72.8	28.2	5410.3	2.85
	227	0.0	67.3	29.7	6305.0	2.99
	234	0.1	62.1	30.9	6401.9	3.42
	242	0.0	67.7	29.6	5803.0	4.12
September	248	10.4	73.5	29.1	5109.6	2.44
	255	11.9	73.4	28.6	5089.0	3.07
	263	64.1	77.0	28.3	4526.8	2.60
	269	2.6	74.9	29.7	5061.4	1.77
	277	93.1	73.4	28.6	3972.0	3.22
October	284	6.1	76.3	29.0	5288.7	2.59
	290	4.6	80.0	28.3	4709.9	1.85
	297	39.1	79.7	28.4	5520.0	2.68
	304	38.3	79.3	28.2	5028.3	2.57
November	311	13.9	78.6	28.3	4676.7	1.85
Average*		**319.5**	**72.1**	**29.0**	**5298.0**	**3.02**
Total*		**402.2**	**70.3**	**29.5**	**5588.1**	**3.50**

*For rainfall, the shown values correspond to their accumulated. For the other meteorological variables, these values correspond to their average.

than *P. pinnatum* in the two periods. Both incidence and severity in *E. cyclocarpum* increased in the rainy season, registering a mean incidence of 83.9%, in contrast with the mean incidence during the dry period (44.3%). Mean severity in rainy months was 11.3%, while in dry months was 8.2%.

Table 3. Mean of incidence and severity of foliar Anthracnose, and defoliation in *E. cyclocarpum* and *P. pinnatum* in two follow-up periods. Universidad del Magdalena, Santa Marta, Colombia. 2016.

Variables (%)	*E. cyclocarpum*		*P. pinnatum*	
	Dry (March-June)	Rainy (July-November)	Dry (March-June)	Rainy (July-November)
Incidence	44.3	83.9	35.8	19.9
Severity	8.2	11.3	0.6	0.3
Defoliation	32.6	19.8	22.6	17.7

In *E. cyclocarpum*, from 26-03-2016 (day 86) and until 04-06-2016 (day 156), a decrease in the incidence of the disease was observed (from 68.0 to 28.8%). Starting on day 156, a linear growth was evidenced until 04-09-2016 (day 248), when the disease reached 100% and stabilized until the end of monitoring (06-11-2016; Figure 2A). The disease severity had a similar behavior; it decreased in the first six weeks, reaching a minimum percentage on 04-07-2016 (day 191) with 2.0%. Subsequently, from day 198 to the end of monitoring on 06-11-2016 (day 311), a linear increase was recorded, with a maximum value of 19.6%, considered moderate (Figure 2B).

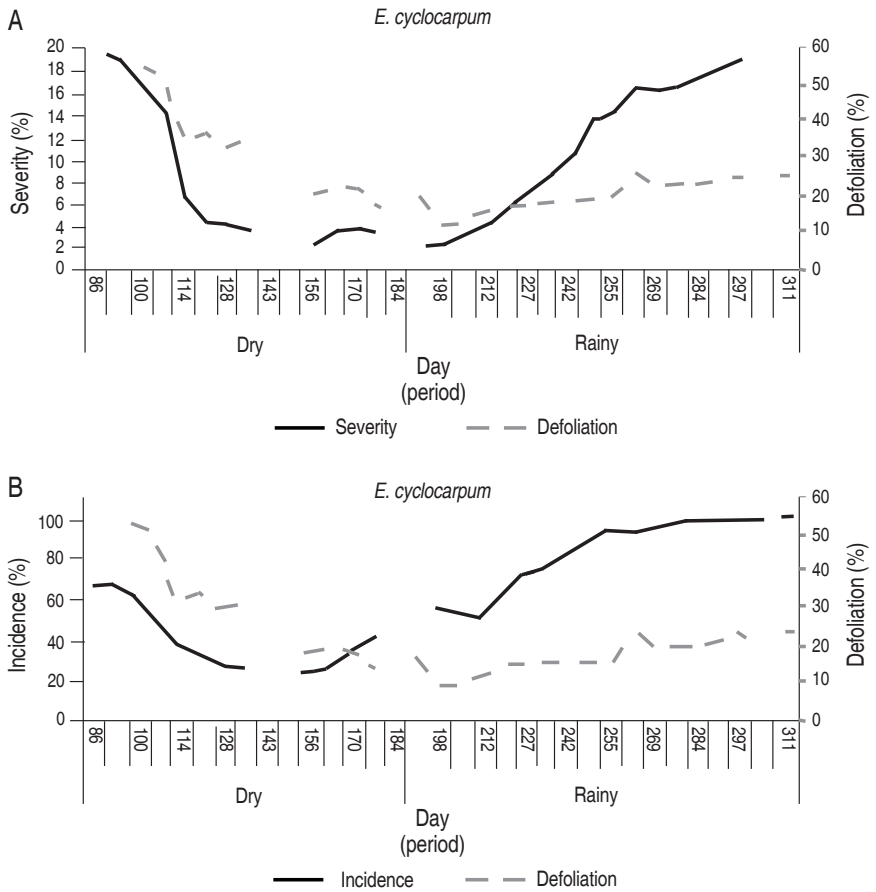

Figure 2. Development curve of foliar Anthracnose associated with *Colletotrichum* spp. in *E. cyclocarpum*. A. Severity and defoliation. B. Incidence and defoliation.

Defoliation in *E. cyclocarpum* was strong in the first weeks, with 54.6% in 09-04-2016 (day 100), and progressively decreased until 16-07-2016 (day 198), with values of 12.3 %. From this date, the defoliation increased until the end of the follow-up period, reaching moderate values of 25.5% (Figure 2).

According to Spearman's correlation analysis (Table 4), the severity of the disease showed a significant relationship with defoliation (correlation=0.431; *P*<0.05) indicating that as the affected leaf area increases, the greater the induction of leaflet defoliations. The high correlation between incidence and severity (correlation=0.807; *P<0.05*) explains that in higher inoculum pressure, new leaflets are infected.

Table 4. Correlation coefficients between epidemiological variables of foliar Anthracnose and defoliation in *E. cyclocarpum*.

	Defoliation	Incidence
Incidence	-0.073	——
Severity	0.431*	0.807*

*Significant correlation (*P<0.05*).

In *P. pinnatum*, the incidence and severity of the disease decreased from 26-03-2016 (day 86) to 14-05-2016 (day 135); the incidence was reduced from 68.9 to 15.0%, while the severity decreased from 1.3 to 0.2%. From day 135, there was a slight increase in the values of both variables, presenting 25.0 and 0.3% of incidence and severity on day 311 (11-06-2016). Defoliation presented

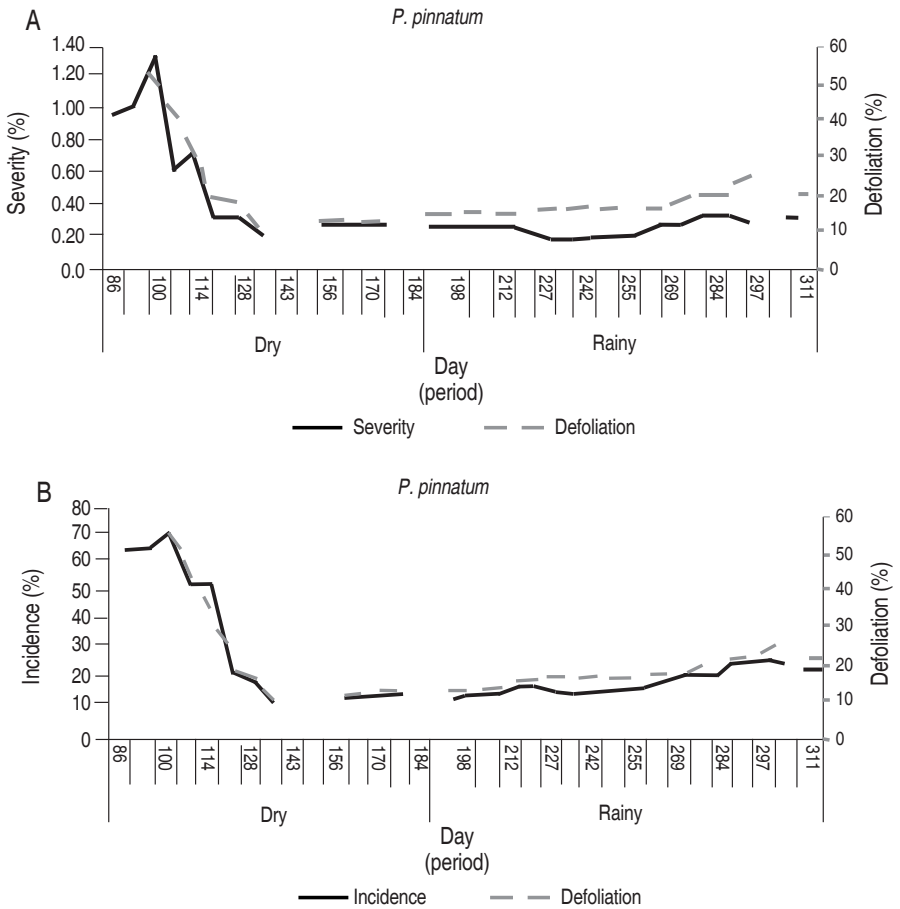

Figure 3. Development curve of the foliar Anthracnose associated with *Colletotrichum* spp. in *P. pinnatum*. A. Severity and defoliation. B. Incidence and defoliation.

a similar behavior to the other two variables, decreasing to 11.6% on day 135 (14-05-2016). From that date, it showed a slight increase, registering 20.7% at the end of the follow-up period (Figure 3).

According to the correlation analysis, there was a significant direct relationship between incidence and severity in *P. pinnatum* with defoliation, and between incidence and severity (Table 5).

Table 5. Correlation between epidemiological variables of foliar Anthracnose and defoliation in *P. pinnatum*.

	Defoliation	Incidence
Incidence	0.867*	——
Severity	0.540*	0.692*

*Significant correlation (*P<0.05*).

The evaluated epidemiological variables indicated that *E. cyclocarpum* has a greater susceptibility to *Colletotrichum* spp., compared to *P. pinnatum*. One explanation for the differences in the amount of disease present in each forest species may be related to the production of secondary metabolites and the anatomy of their leaves. In *Platymisicium* species, the presence of flavonoids, isoflavonoids and other compounds with antifungal and cytotoxic properties has been reported (Cardoso-Lopes *et al*., 2008; Cuellar *et al*., 2020), although plant metabolites produced by *E. cyclocarpum* may also have antifungal activity (Pacheco *et al.,* 2012; Biabiany *et al*., 2013). Arambarri *et al.* (2006) observed the presence of conspicuous epicuticular waxes in *Enterolobium* spp., a common feature of species adapted to dry environments, which can confer resistance to penetrating pathogens. Regarding *P. pinnatum*, de Enrech and Agostini (1987) indicated that the cuticle of this species presents a greater thickness, compared to other *Platymiscium* species. It is known that a greater thickness in the cuticle, as well as the presence of additional waxes, confers greater resistance to the penetration of fungi; however, it is worthy to mention that many pathogens can establish infections in plants with a considerable cuticle thickness (Freeman and Battie, 2008). In this research, the characteristics described for the leaves of both forest species did not prevent the development of the disease. It would be necessary to carry out studies on the physical and chemical composition of the leaves in these forest trees, as well as their relationship with resistance to *Colletotrichum* species.

The development of the disease depended fundamentally on the phenological development of the hosts since this defines successive sprouts and defoliation that, in turn, influence the diseases values over time. Initially, in the available leaf area, the disease values increase, being more evident with the first defoliation; however, as defoliation becomes widespread, the affected tissue is removed and the amount of initial disease decreases. In other words, the reduction in the amount of disease (incidence and severity) is mainly due to the loss of the plant organ (defoliation) and the appearance of new healthy tissue. This behavior has been used to establish management techniques for foliar Anthracnose where, through artificial defoliation, the severity of the disease is reduced (Guyot *et al*., 2005). However, a progressive increase in defoliation is an indicator of disease severity (Guyot *et al*., 2005; Huertas-Palacios *et al*., 2009).

The real effect of the fungus on defoliation is not understood nor if this is a plant defense mechanism against infection. In this research, the highest defoliation values, in both species, were recorded during the driest months. *E. cyclocarpum* is a semi-deciduous species that loses part of its foliage during the driest months, beginning defoliation at the end of the rainy season. On the other hand, the increase in foliage occurs when rainfall increases after the end of the dry season (Rocha *et al*., 2018). *P. pinnatum* is deciduous during the dry season, where trees can lose between 50 and 80% of their foliage, and as rainfall increases, the fall of the foliage decreases (Gómez, 2010). It is recommended to evaluate the effect of foliar Anthracnose in the defoliation of these forest species in future investigations.

Development rate and area under the disease progress curve (*AUDPC*)

In *E. cyclocarpum*, during the dry period (March to June), the foliar Anthracnose presented a development rate of 0.136 units week^{-1}. Starting with the rains of July, and increasing in September and October, *r* reached 0.187 units week^{-1}. The *AUDPC* was lower in the first

period (dry), with 51 units week^{-1}, compared to 186 units week^{-1} obtained during the rainy period (Table 6). These results indicated a more rapid development of the disease during the second half of the year.

In *P. pinnatum*, the disease presented a development rate of 0.107 units week^{-1} during the first semester of the year. In the rainiest months, the development rate was lower (0.016 units week^{-1}). The *AUDPC* indicated that foliar Anthracnose was higher in the months with less rainfall, registering a value of 4 units week^{-1}, while in the second period the *AUDPC* was 2 units week^{-1} (Table 6). In this host, different behavior of the disease was registered to that obtained in *E. cyclocarpum*, which makes to consider other factors than the environment.

Table 6. Development rate and area under the progress curve of foliar Anthracnose associated with *Colletotrichum* spp. in *E. cyclocarpum* and *P. pinnatum* for two follow-up periods.

	Dry period (March – June)		Rainy period (July – November)	
	Development rate (*r*) (units week^{-1})	***AUDPC* (units week^{-1})**	**Development rate (*r*) (units week^{-1})**	***AUDPC* (units week^{-1})**
E. cyclocarpum	0.136	51	0.187	186
P. pinnatum	0.107	4	0.016	2

In this study, *P. pinnatum* presented lower development of foliar Anthracnose, especially when the climatic conditions were more favorable for the synthesis. In contrast, *E. cyclocarpum* was more susceptible to the action of the pathogen, observing a greater progression of the disease during periods where conditions of high humidity prevail. This greater disease progress during the rainy season agrees with studies in other pathosystems, including *Colletotrichum* species (Huertas-Palacios *et al*., 2009; Moral *et al*., 2012; Miles *et al*., 2013; Lima *et al*., 2015).

Effect of meteorological variables on the development of the disease

In *E. cyclocarpum*, the incidence of foliar Anthracnose was inversely correlated with mean temperature (*P<0.05*). Although the analysis showed a significant negative correlation with solar radiation and wind speed, and a positive correlation with relative humidity, the coefficients were not high. For severity, a significant negative correlation with mean temperature was observed, but with a low coefficient (-0.391; Figure 4-5; Table 7). In *P. pinnatum*, this type of analysis did not allow to identify significant correlations between epidemiological variables of the disease and meteorological variables (Figure 5-7; Table 7).

The multiple regression analysis by ordinary least squares confirmed that mean temperature was the only variable related to the incidence of foliar Anthracnose in *E. cyclocarpum*, explaining the incidence behavior by 39.72% with the whole model (Table 8). In the highest temperature values (between March and June) with an average of 30 °C (Table 2), the incidence remained at values close to 45% (Figure 2B); however, the temperature decreased slightly, except for certain events with maximum peaks, coinciding with the increase in incidence. On the other hand, severity could not be explained from the behavior of any of the analyzed meteorological variables. This raises the hypothesis that there are factors intrinsic to the pathogen or the host that influence a lesser or greater aggressiveness of the disease, under the meteorological conditions of the study area.

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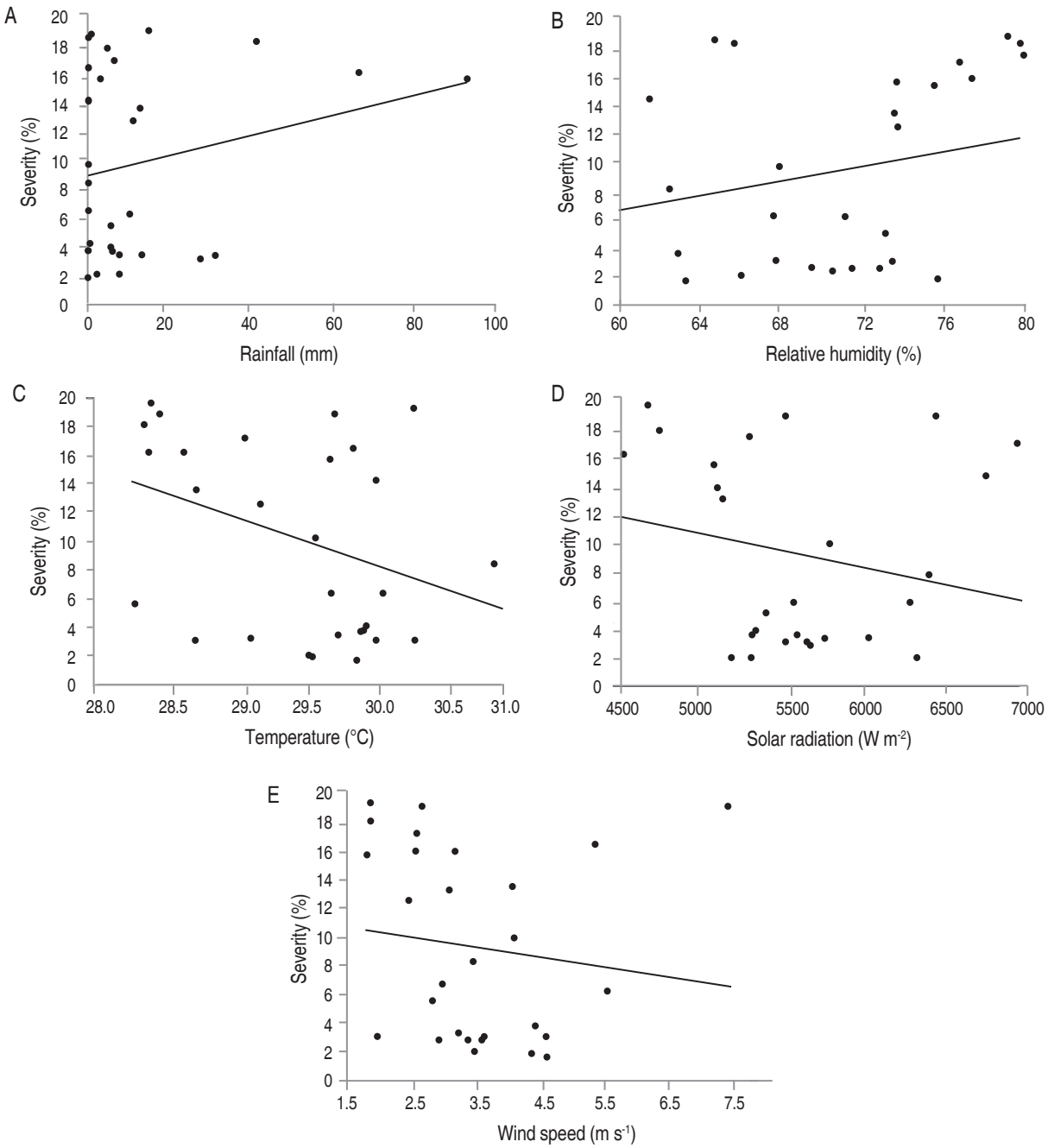

Figure 4. Relationship between the meteorological variables with the severity of foliar Anthracnose in *E. cyclocarpum*. Severity with (A) rainfall, (B) relative humidity, (C) mean temperature, (D) solar radiation, and (E) wind speed.

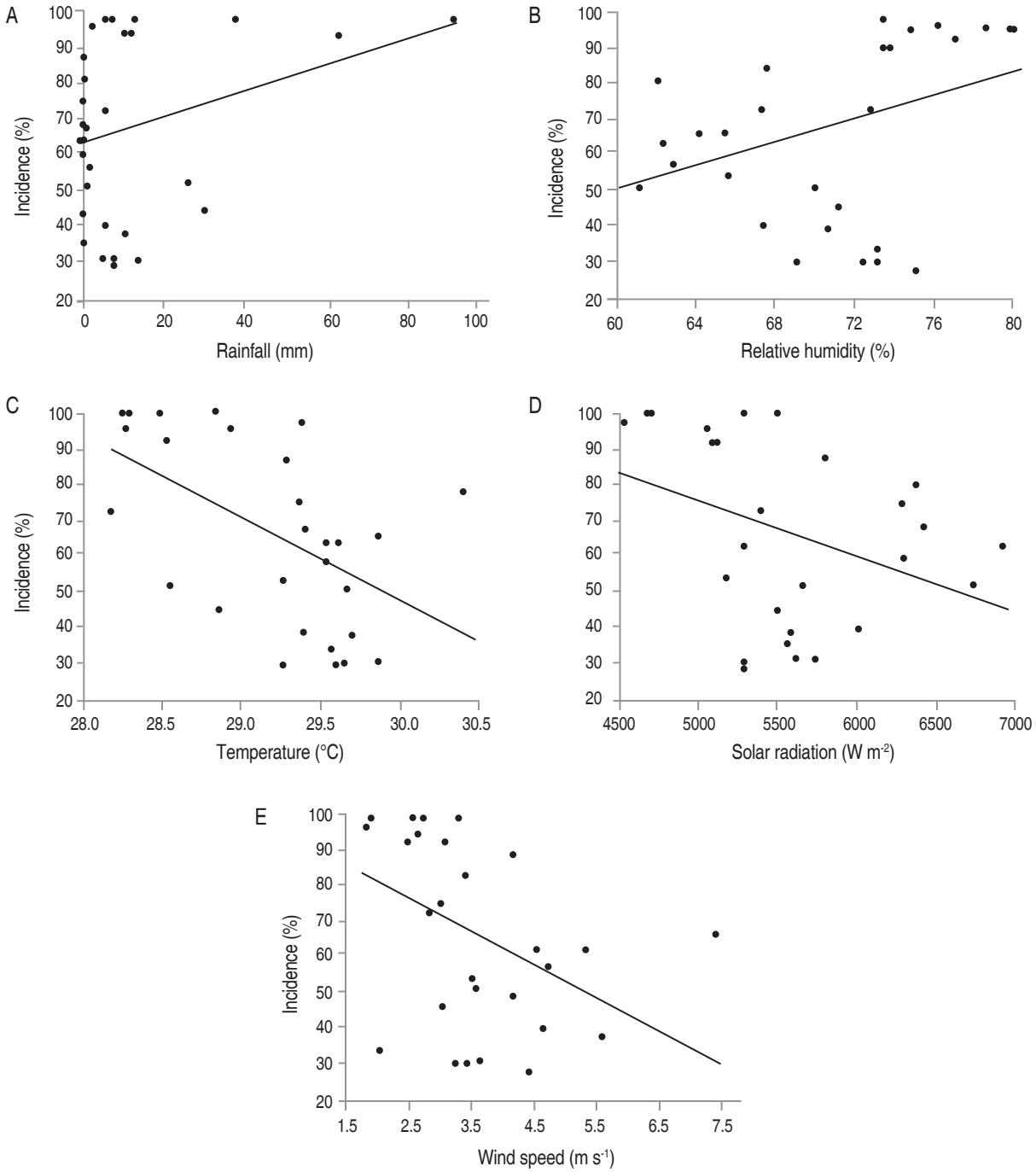

Figure 5. Relationship between the meteorological variables with the incidence of foliar Anthracnose in *E. cyclocarpum.* Incidence with (A) rainfall, (B) relative humidity, (C) average temperature, (D) solar radiation, and (E) wind speed.

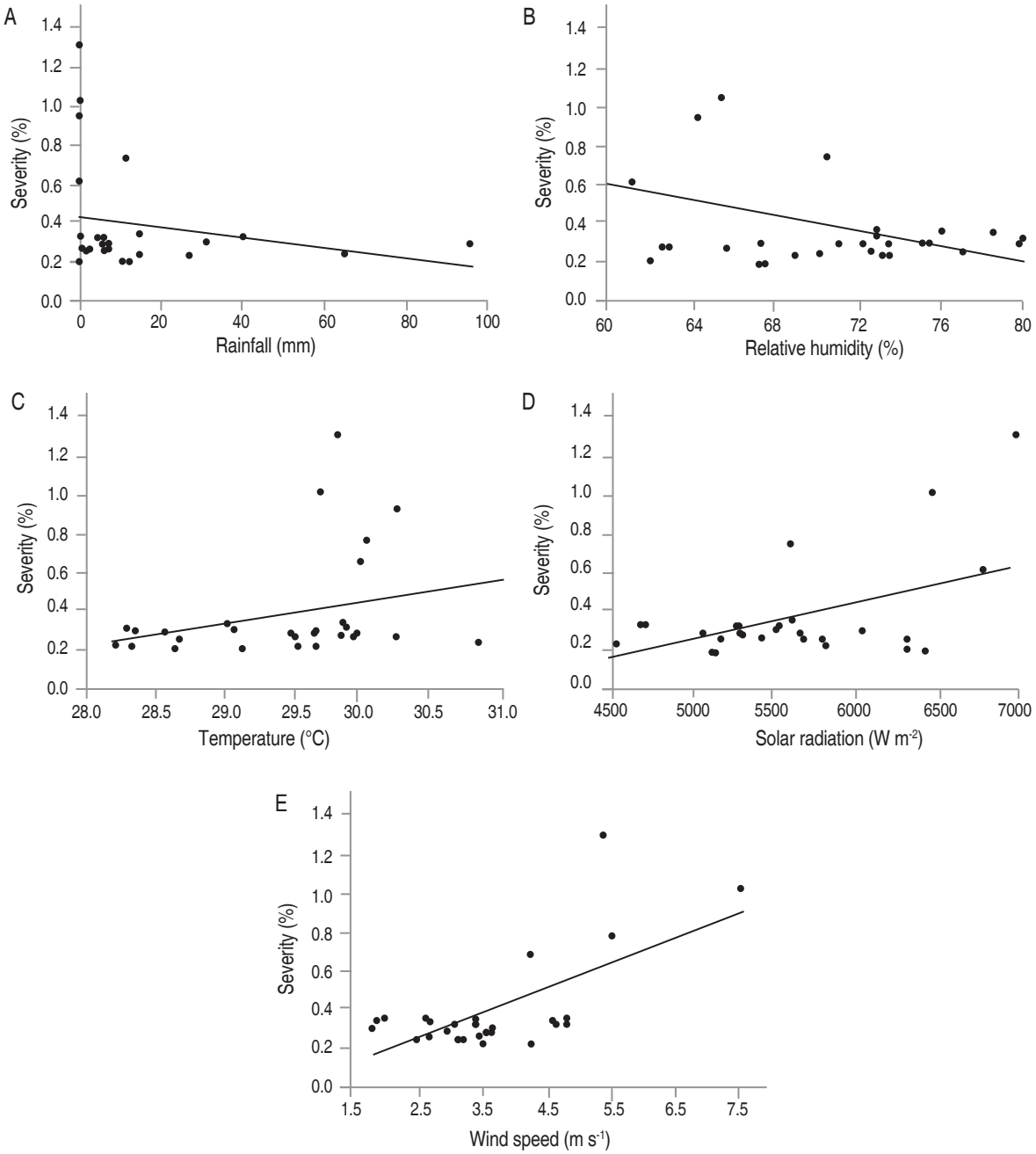

Figure 6. Relationship between the meteorological variables with the severity of foliar Anthracnose in *P. pinnatum*. The severity with (A) rainfall, (B) relative humidity, (C) mean temperature, (D) solar radiation, and (E) wind speed.

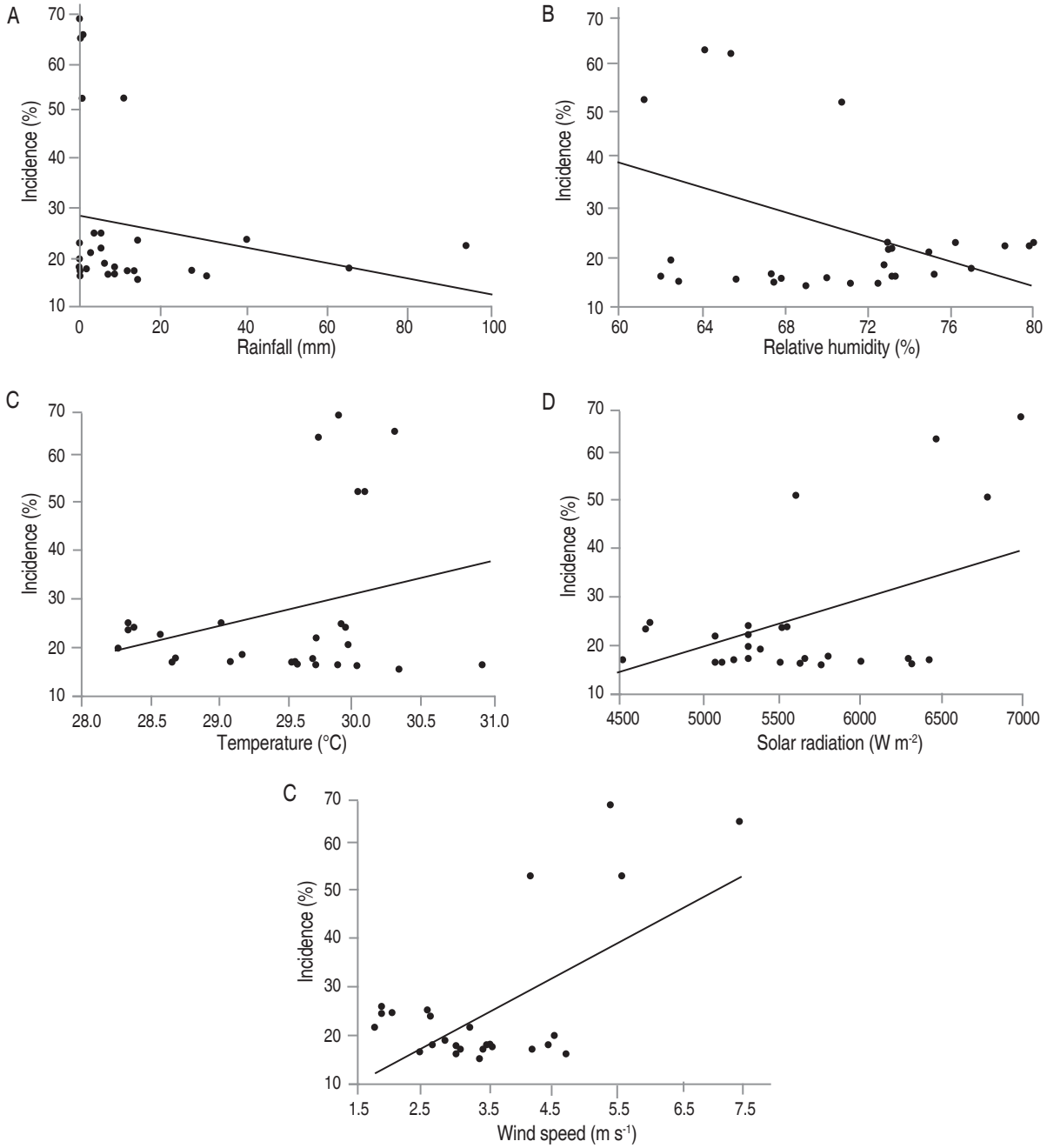

Figure 7. Relationship between the meteorological variables with the incidence of foliar Anthracnose in *P. pinnatum*. Incidence with (A) rainfall, (B) relative humidity, (C) mean temperature, (D) solar radiation, and (E) wind speed.

Table 7. Correlation between the meteorological variables and diseases values (incidence and severity) of foliar Anthracnose in the foliage of *E. cyclocarpum* and *P. pinnatum*, recorded from 26-03-2016 to 06-11-2016. (n = 29).

Meteorological variable	*E. cyclocarpum*		*P. pinnatum*	
	Incidence	Severity	Incidence	Severity
Rainfall	0.1644	0.0836	-0.1670	-0.0819
Relative humidity	0.4399*	0.3668	0.1269	0.0529
Mean temperature	-0.6345*	-0.3907*	-0.1143	0.1111
Solar radiation	-0.4628*	-0.2304	-0.0486	0.1253
Wind speed	-0.5382*	-0.3582	-0.0572	0.1593

*Significant correlation (*P*<0.05).

Table 8. Multiple regression between meteorological variables and the incidence of *Colletotrichum* spp. in the foliage of *E. cyclocarpum*, registered from 26-03-2016 to 06-11-2016.

Variable		Estimation	*P-value*
Rainfall		0.000214	0.9379
Relative humidity		-0.013140	0.3607
Average temperature		-0.219628	0.0226*
Solar radiation		-0.000023	0.8423
Wind speed		-0.058586	0.2165
Multiple regression	R = 0.6302 R^2 = 0.3972 Adjusted R^2 = 0.2602	8.38838	0.0370*

*Significant variable (*P*<0.05).

In *P. pinnatum*, the multiple regression analysis indicated a relationship of incidence and severity of foliar Anthracnose with wind speed, explained in 44.59 and 50.29% of its behavior with the whole model, respectively (Tables 9 and 10). Figures 6E and 7E show that the maximum values of wind speed, which correspond to March-April, coincided with the highest values recorded in both incidence and severity. Nevertheless, it is noted that these variables were not correlated (Table 7).

The temperature was the only variable that influenced the development of the disease in *E. cyclocarpum*, showing a negative correlation. Although an optimal temperature range of 25-30 °C is proposed for *Colletotrichum* spp. (Miles *et al*., 2013), the different fungal species present specific requirements (Lima *et al*., 2015). In this study, when the temperature was more favorable for the activity of the pathogen, an increase in the disease was evidenced. Nonetheless, to better understand the effect of temperature on the development of the disease, it would be necessary to carry out complementary research on the specific optimum temperature of the *Colletotrichum* species found in the forests of the Caribbean region.

In other interactions, in addition to temperature, relative humidity affects the expression of disease, such as those observed in Anthracnose caused by *Colletotrichum* spp. in avocado (Márquez, 2016), and

in mango inflorescences (Páez *et al*., 2003). Likewise, correlations have been described between solar radiation and the number of lesions of *C. gloesporioides* in *Stylosanthes scabra* (Pangga *et al*., 2011), on the survival and production of conidia of *Colletotrichum acutatum* (Fracarolli *et al*., 2016), or the beginning of *Colletotrichum lindemuthianum* infections in beans (Pérez-Vega *et al*., 2010). Nevertheless, in the present study, this variable was not determining for the disease behavior.

Table 9. Multiple regression between meteorological variables and the incidence of *Colletotrichum* spp. in the foliage of *P. pinnatum*, registered from 26-03-2016 to 06-11-2016.

Variable		Estimation	*P-value*
Rainfall		0.000248	0.8681
Relative humidity		0.005172	0.5064
Average temperature		-0.038458	0.4382
Solar radiation		0.000093	0.1541
Wind speed		0.067594	0.0131*
Multiple regression	R = 0.6678 R^2 = 0.4459 Adjusted R^2 = 0.3199	0.259038	0.0169*

*Significant variable (*P*<0.05)

Table 10. Multiple regression between meteorological variables and the severity of *Colletotrichum* spp. in the foliage of *P. pinnatum*, registered from 26-03-2016 to 06-11-2016.

Variable		Estimation	p-value
Rainfall		0.000004	0.8664
Relative humidity		0.000084	0.5177
Average temperature		-0.000698	0.3986
Solar radiation		0.000002	0.1234
Wind speed		0.001245	0.0068*
Multiple regression	R = 0.7091 R^2 = 0.5029 Adjusted R^2 = 0.3899	0.004447	0.0059*

*Significant variable (*P*<0.05)

For *P. pinnatum*, although the regression analysis indicated a relation between wind speed and level of disease (incidence and severity), the correlation was not significant. In contrast, disease incidence in *E. cyclocarpum* was correlated with wind speed. The effect of wind speed on diseases caused by *Colletotrichum* spp. is mainly related as a complementary mechanism of spore dissemination (Siddiqui and Ali, 2014). However, the correlation between disease increase and wind speed is not clear. According to Guyot *et al.* (2005), although strong winds can favor an increase in infections, this phenomenon does not necessarily produce a notable dispersal of spores.

In this study, no relationship was found between rainfall and disease progress under conditions of the Universidad del Magdalena campus. Similar results have been observed in the epidemiology of *Colletotrichum* spp. in mango (Páez *et al.,* 2003) and in *Heliconias* cultivars (López-Vásquez *et al.,* 2013). Some studies show that high rainfall does not necessarily favor the dispersal of *Colletotrichum* spp. spores. (Guyot *et al*., 2005). Even heavy rainfall can have a negative effect on the severity of *Colletotrichum* spp., due to the washing of the inoculum caused by the rains (Huertas-Palacios *et al.,* 2009).

CONCLUSIONS

Foliar Anthracnose associated with *Colletotrichum* spp. in forest species varied with the period of the year and the host. During the months with the highest rainfall, the highest incidence and severity values were presented in *E. cyclocarpum*; however, in *P. pinnatum*, the maximum values were observed during the dry period. Likewise, the values of the development rate and the area under the disease progress curve indicated different behaviors in each pathosystem.

The progress of the disease was related to relative humidity, temperature, solar radiation and wind speed for *E. cyclocarpum*. In *P. pinnatum*, no correlation was found between environmental factors and disease level. It is highlighted that the phenological cycles of the host, which define defoliation and successive regrowth, influenced the initiation and development of multiple infective processes. It would be necessary to clarify the influence of the disease/pathogen presence in the host's defoliation in further investigations.

Finally, the initial hypothesis was confirmed, since the behavior of foliar anthracnose caused by *Colletotrichum* spp. varied according to the forest species. This research provides the first epidemiological studies of *Colletotrichum* spp. associated with forest trees in the Colombian Caribbean region.

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Quality of tomato (*Solanum lycopersicum* L.) fruits inoculated with *Escherichia coli* under different storage conditions

UNIVERSIDAD NACIONAL DE COLOMBIA

Calidad de frutos de tomate (*Solanum lycopersicum* L.) inoculados con *Escherichia coli* en diferentes condiciones de almacenamiento

https://doi.org/10.15446/rfnam.v75n1.95626

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ABSTRACT

Keywords:
Bacterial adherence
Color
Firmness
Storage temperature
Tomato

The study evaluated the effect of storage temperatures of 7 and 22 °C for 168 h on tomatoes (Charleston cv.) inoculated with 10^7 CFU mL^{-1} of the enterotoxigenic *Escherichia coli* pathogroup (ETEC) strain on color indexes (hue angle, *h*°, and chroma, *C**), firmness, titratable acidity (% citric acid), ascorbic acid, total soluble sugars and reducing sugars (glucose, fructose, and sucrose). ETEC survived with populations of 7 and 9.2 Log CFU g^{-1} at 7 and 22 °C, respectively until 120 h. Bacterial adherence and colonization under both storage conditions were confirmed by scanning electron microscopy. The index *C** and ascorbic acid had higher values at 22 °C, while the parameters *h*°, firmness, and citric acid had lower values at the same storage temperature. At 7 °C, the concentration of total soluble sugars was affected; glucose and fructose showed lower values (0.054 and 0.057 g 100 g^{-1}, respectively). Finally, the inoculated fruits exhibited significant differences in the parameters of consumer preference of fresh tomatoes such as color, firmness, sugars, and organic acids, which were affected depending on the storage temperature.

RESUMEN

Palabras clave:
Adherencia bacteriana
Color
Firmeza
Temperatura de almacenamiento
Tomate

El estudio evaluó el efecto de temperaturas de almacenamiento de 7 y 22 °C durante 168 h en tomates (cv. Charleston) inoculados con 10^7 UFC mL^{-1} de la cepa *Escherichia coli* del patogrupo enterotoxigénico (ECET) sobre índices de color (ángulo de tono, *h*° y croma, *C**) firmeza, acidez titulable (% ácido cítrico), ácido ascórbico, azúcares solubles totales y azúcares reductores (glucosa, fructosa y sacarosa). ECET sobrevivió con poblaciones de 7 y 9.2 Log UFC g^{-1} a 7 y 22 °C, respectivamente, hasta las 120 h. La adherencia y colonización bacteriana en ambas condiciones de almacenamiento se confirmaron mediante microscopía electrónica de barrido. El índice *C** y el ácido ascórbico tuvieron valores más altos a 22 °C, mientras que el parámetro *h*°, firmeza y ácido cítrico tuvieron valores más bajos a la misma temperatura de almacenamiento. La temperatura de 7 °C afectó la concentración de azúcares solubles totales; glucosa y fructosa con valores menores (0,054 y 0,057 g 100 g^{-1}, respectivamente). Finalmente, los frutos inoculados exhibieron diferencias significativas en los parámetros de preferencia del consumidor de tomates frescos como color, firmeza, azúcares y ácidos orgánicos, los cuales se vieron afectadas dependiendo de la temperatura de almacenamiento.

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In Mexico, tomato production is increasing. In 2017, the harvested volume was more than 631 thousand tons. Tomato is in the first place of exported agricultural produce (SIAP, 2018). However, safety specifications for fresh produce can put this progressive preference for the vegetable at risk because production practices and post-harvest handling conditions occur in environments that favor the development of pathogenic and deteriorative microorganisms (Orozco *et al*., 2008).

Firmness and color are the most significant quality attributes of fresh tomatoes used by growers and consumers as selection criteria (Khairi *et al*., 2015), while the content of sugars and organic acids are important factors that determine flavor (Agius *et al*., 2018). During post-harvest, these attributes are affected by storage conditions, such as temperature and relative humidity. Low temperatures can substantially increase shelf life by slowing down the fruit ripening process and reducing microbial activity (Guatam *et al*., 2015). A wide variety of microbial species can lodge on the surface of the product adhering and forming biofilms, rendering the washing and disinfection processes ineffective (Iturriaga *et al*., 2003). The chance of bacterial contamination of fruits is high, given that the content of water and nutrients support bacterial growth, besides micro-rough texture facilitating bacterial adhesion and establishment (Torres-Aguilar *et al*., 2016).

Adherence, as a survival mechanism, allows enteropathogens of the genera *Escherichia*, *Salmonella,* and *Shigella* to remain on tomatoes, lettuce, peach, spinach, broccoli, alfalfa, and apple and orange juice, whose consumption is associated with outbreaks of gastrointestinal disease (Scallan *et al*., 2011). This becomes even more important when these fresh vegetables are eaten raw in salads or juices. In developing countries, enterotoxigenic *Escherichia coli* (ETEC) is the second pathogroup that causes gastroenteritis in children during their first years of life (Walker *et al*., 2007). ETEC is also linked to traveler's diarrhea caused by lack of hygiene and fecal contamination (Loc *et al*., 2014). ETEC's virulence factors are the heat-labile toxin and the heat-stable toxin (Fleckenstein *et al*., 2010). Furthermore, these bacteria can survive in a variety of environments, such as rivers, drinking water, irrigation water, and fresh vegetables (MacDonald *et al*., 2015).

There are several studies in tomatoes concerning *E. coli* contamination during production, harvest, and commercialization (Gómez-Aldapa *et al*., 2013). However, there are few references on the relation of quality attributes and survival of *E. coli* on fruits stored at different temperatures. For this reason, this study aimed to evaluate the effect of 7 and 22 °C temperatures for 168 h of storage on tomatoes inoculated with enterotoxigenic *E. coli* (ETEC) as well as color indexes (hue angle, $h°$, and chroma, C^*), firmness, titratable acidity (% citric acid), ascorbic acid, total soluble sugars and reducing sugars (glucose, fructose, and sucrose).

MATERIALS AND METHODS

Plant material

The material used consisted of fruits of tomato (*Solanum lycopersicum* L.) Charleston cv. harvested at 5 degrees (luminous red), according to the color table of the Department of Agriculture of the United States (USDA, 2017), and collected from the central part of the hydroponic type greenhouse in Aquixtla, Puebla, Mexico. The fruits were transported and maintained in refrigeration in a cooler for less than 12 h before laboratory analyses started.

Studied microorganism

The enterotoxigenic *E. coli* (ETEC) pathogroup strain was isolated from tomato fruits and the soil of the greenhouse (Luna-Guevara *et al*., 2012). This strain was identified by IMViC biochemical tests (indol, methyl red, Voges-Proskauer, and Simmons citrate) and tests of the automated system VITEK (Biomeriux, Mexico) and confirmed by polymerase chain reaction (PCR) with amplification of the IngA gene (Luna-Guevara *et al*., 2015).

Fruit inoculation and storage procedures

Intact fruits were washed and disinfected with a 70% (v/v) ethanol solution, rinsed with sterile water, and dried. Fruits were inoculated by immersion for 10 minutes in a 10^7 CFU mL^{-1} bacterial culture with an optical density (OD) 1.1 at a wavelength of 620 nm. Inoculation was confirmed by plate count. The inoculated fruits were deposited in plastic hermetically sealed containers (25x10x20 cm) and stored at two temperatures (T): 7 and 22 °C with an interior relative humidity (RH) of 60% adjusted with saturated sodium bromide salts (Iturriaga

et al., 2007). Interior T and RH of the containers were monitored with an environment datalogger (HOBO H08-004-02, Onset Pro Computer Corporation, MA, USA).

Microbial counts

Approximately, 2 mm of tissue was taken from the epicarp and mesocarp at the equatorial region of the fruit. This tissue was homogenized in 50 mL of 0.1% peptone water. Serial dilutions (10^4 to 10^7) were made and spread on plates in trypticase soy agar (Bioxon, Mexico) for enumeration of *E. coli*. The plates were incubated at 37 °C for 24 h and the presence of *E. coli* was confirmed using conventional IMViC biochemical tests. This procedure was repeated in triplicate at 1.5, 24, 72, 120, and 168 h of storage.

Observation of adherence and colonization

Micrographs of the inoculated fruits stored for 1.5 up to 168 h were prepared for examination with a scanning electron microscopy (SEM) following the procedure proposed by Sun *et al*. (2016) with some modifications. The samples were observed in a scanning electron microscope (JEOL, JSM-6390, MA, USA) 10-15 kV range of operation.

Physical tests on fruits stored at different temperatures

Color. The color parameters CIE L*, a*, and b* were determined in triplicate at the equatorial zone of five fruits using a colorimeter (Hunterlab, ColorFlex-45) (Pathare *et al*., 2013). With these chromatids, hue angle (*h*°, related to reds and greens) and color purity (*C**, chroma) were calculated.

Firmness. The firmness of intact fruits was determined as the force required for a 6 mm diameter cylindrical TA-212 awl and a texturometer TAXT plus (Texture Technologies, Surrey, UK) to penetrate 5 mm at a velocity of 1.0 mm s^{-1}. The results were expressed in Newtons (N) and each reported value of firmness represents the mean of three individual measurements taken on three tomato samples.

Titratable acidity. The acidity of the juice extracted from 10 g of fruit was evaluated by titration with NaOH at 0.1 N until reaching a pH of 8.1. The result was expressed as a percentage of citric acid (% citric acid) (Horwitz, 2000).

Chemical properties of fruits stored at different temperatures

T*otal soluble (TSS) and reducing sugars (RS).*

For both types of sugars, 100 mg of sample was extracted and incubated in periods of 10 min five times successively in 80% ethanol at 70 °C. The supernatants were evaporated at 50 °C, dissolved in 1 mL of distilled water, and stored at -20 °C until analysis. The TSS were determined following the Antrona method proposed by Montreuil *et al*. (1997) and RS were quantified using the method described by Scholes *et al*. (1994). The calculations used standard calibration curves, which were prepared previously for each of the sugars, results were expressed in g ·100 g^{-1} fresh weight (f.w.).

Vitamin C. Total ascorbic acid was analyzed by the spectrophotometer method described by Noctor and Foyer (1998) using 100 mg fruit. Absorbance readings were carried out in a spectrophotometer UV/Vis (JEYWAY 7305, ThermoLab, USA) at a wavelength of 265 nm before and after adding 20 µL ascorbate oxidase (0.05 U). The blank consisted of a 120 mM sodium phosphate buffer, pH 5.6.

Statistical analysis

The experimental data were analyzed statistically by a completely randomized experimental design with three replications. For the set of treatments, an analysis of variance (ANOVA) was performed and means were compared with an honest significant difference of $P<0.05$ (Tukey). The Statistical Analysis System (SAS), version 9.0 (SAS, 2002) was used.

RESULTS AND DISCUSSION

Effects of storage conditions on *Escherichia coli* (ETEC) growth

Effect of storage conditions on *E. coli* (ETEC) survival on inoculated tomato fruit was significant ($P<0.05$), the bacterial populations were 7 and 9.2 Log CFU g^{-1} at 7 and 22 °C, respectively, after 120 h of storage. This behavior is similar to that reported by Gómez-Aldapa *et al.* (2013), who reported the growth of *E. coli* (enterotoxigenic) in mung bean sprouts and the growth of *E. coli* on foods stored in refrigeration (Kothe *et al*., 2019).

The *E. coli* (ETEC) counts of CFU g^{-1} recorded at refrigeration temperature (Figure 1) evidence the psychrotrophic capacity of this microorganism to grow in fresh food products (Pothakos *et al*., 2012; Keshri *et al*., 2019), and its permanence under this condition indicates that it favors ETEC survival. For this reason,

the consumption of fresh tomatoes makes it necessary to consider preventive measures to maintain the safety of the fruit and avoid it from becoming a reservoir of enterobacteria, including *E. coli*, which can produce gastrointestinal disorders (Mansan-Almeida *et al*., 2013).

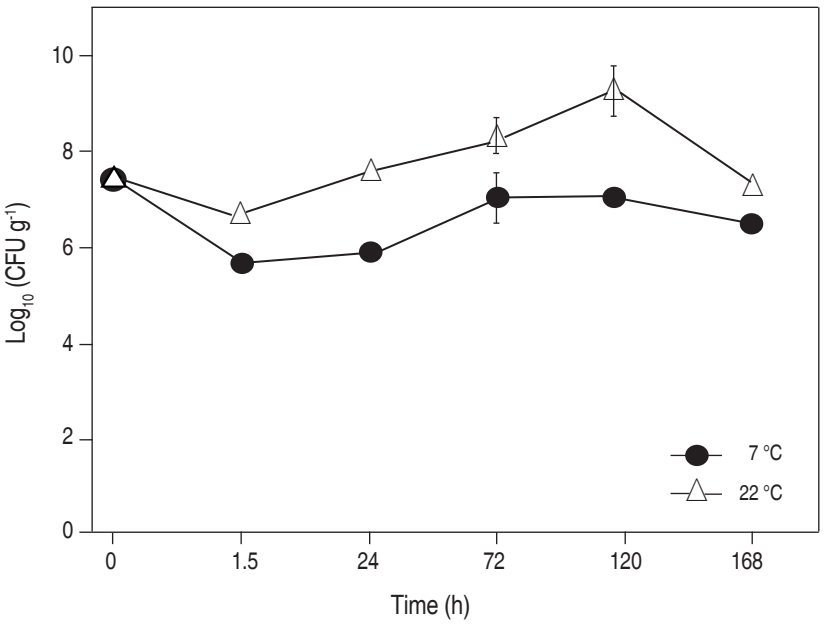

Figure 1. *Escherichia coli* (ETEC) survival at 7 and 22 °C on tomato fruits.

Adherence of ETEC on tomatoes stored at different temperatures

The pericarp of fruits stored at 7 and 22 °C showed growth of ETEC from 1.5 h of storage (Figures 2A and 2B, respectively). After 72 h post-inoculation, adherence of *E. coli* was notable (Figures 2C and 2D). According to Shaw *et al*. (2011), adherence is carried out through a diffuse mechanism of adhesion mediated mainly by adhesins of the flagella on vegetables. Other reports have shown that *E. coli* can produce biofilms through curli and extracellular matrix (1.5-n-acetyl-D-glucosaminecellulose, cellulose, and colonic acid) on sprouts and tomato roots (Matthysse *et al*., 2008). It has been observed that the growth of enteric pathogens such as ETEC is greater in plant tissue with mechanical damage due to the availability of nutrients (Shaw *et al*., 2011).

Adhesion of the bacteria on the fruit surface under both storage conditions persisted after 168 h is shown in Figures 2E and 2F. The greatest adherence of ETEC at 22 °C (Figure 2E) was related to 120 h of storage (Figure 1). The presence of *E coli*. strains that can form biofilms in both conditions suggests potential health risk for consumers (Liu *et al*., 2013; Corzo-Ariyama *et al*., 2019), given that this contamination may take place during the pre-harvest period, due to the use of a contaminated water supply when cultivating the vegetables, in post-harvest environments, where it may appear after washing and processing the raw material, also due to storage temperatures which allow fast growth of the bacterial (Carter *et al*., 2016).

Effect of storage conditions on color and firmness parameters

Color components of inoculated fruits were affected significantly ($P<0.05$) by storage temperature. The chroma (C^*) value increased as the fruit ripened and showed an increase in color intensity over time. Similar results were described by Navarro-López *et al*. (2012). There was a greater increase in fruits stored at 22 °C, which retained their red color (Table 1). According to López-Camelo and Gómez (2004), the value of C^* influences consumer acceptance of ripe fruits, and thus, inoculated fruits stored at room temperature and in refrigeration are acceptable for the consumer based on color intensity after 168 h. Hue

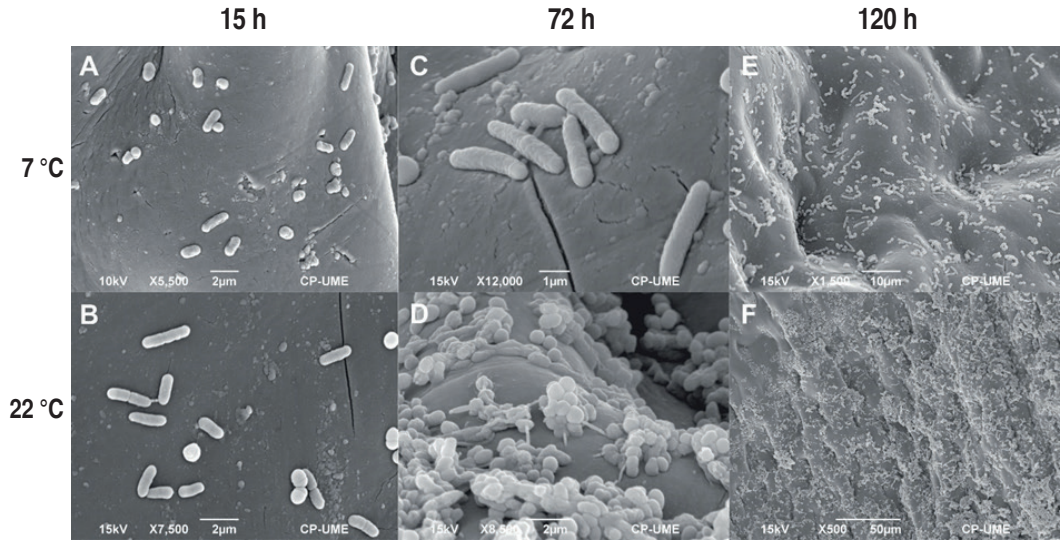

Figure 2. Micrographs of tomato inoculated with *Escherichia coli* (ETEC) at 7 and 22 °C. Micrographs after 1.5 h (A, B), 72 h (C, D), and 168 h (E, F) of storage.

angle, *h*°, decreased with storage time at both temperatures. The fruits stored at 7 °C tended to change color less and had significantly higher values than fruits stored at 22 °C. The decrease in *h*° of fruits stored at 22 °C was more notable at 72 h (Table 1), and even after 168 h, the hue angle of the fruits (24.69) decreased 2.6 times, regarding fruits stored under refrigeration (64.32). The results obtained in this index suggest that the fruits stored at room temperature (22 °C) had a greater color change, this is due to the fact that the tomato fruit being climacteric, their physiological processes continue after harvest and as their maturation progresses, chlorophyll degradation occurs, as well as chromoplast synthesis, promoting the color change from green to red, which shows the presence of pigments such as carotene and lycopene (Pinheiro *et al*., 2013; Carrillo-López and Yahia, 2014; Cherono *et al*., 2018).

Table 1. Physicochemical properties in tomato inoculated with *Escherichia coli* (ETEC), stored at 7 and 22 °C.

Temperature (°C)	Time (h)	Hue angle	Chroma	°Brix	Titratable acidity (% citric acid)	Total soluble sugar	Glucose		Sucrose	Ascorbic acid
						g 100 g^{-1} f.w.				mg 100 g^{-1} f.w.
7	1.5	80.53 a	16.69 c	2.10 b	0.45 a	0.74 b	0.08 a	0.07 a	0.08 a	29.88 b
	72	67.60 b	18.86 b	2.27 b	0.16 b	1.08 ab	0.06 b	0.06 a	0.05 b	54.09 ab
	168	64.32 c	21.02 a	3.37 a	0.19 b	1.18 a	0.07 ab	0.07 a	0.06 b	63.65 a
	LSD ≤ 0.05	0.29	0.03	0.92	0.05	0.36	0.02	0.02	0.01	23.59
22	1.5	76.19 a	19.05 c	2.73 b	0.29 a	1.01 b	0.06 a	0.07 a	0.07 a	54.85 a
	72	64.26 b	22.58 b	3.23 ab	0.16 b	1.42 a	0.05 a	0.06 ab	0.06 a	75.14 a
	168	24.69 c	25. 59 a	4.47 a	0.12 c	1.57 a	0.05 a	0.06 ab	0.06 a	77.10 a
	LSD ≤ 0.05	5.83	2.48	1.53	0.02	0.16	0.02	0.01	0.02	32.76

In each column, means followed by different letters are significantly different, according to the Tukey test ($P \leq 0.05$).
LSD: Least Significant Difference.

Firmness tended to decrease under both conditions of storage temperature, but at 22 °C it was significantly less (P<0.05) up to 168 h (Figure 3). Room temperature can cause a continuous reduction in tomato firmness due to moisture loss through transpiration and enzymatic changes, which can degrade tomato cell wall (Hatami *et al*., 2013; Abiso *et al*., 2015). Firmness reduction is attributed to different factors as losses in cell turgor pressure as well as the cell wall and polysaccharides degradation (Al-Dairi *et al*., 2021). The tendency of the experimental fruit firmness values at 7 °C is similar to that mentioned by Tadesse *et al*. (2015) and Kabir *et al*. (2020), who stated that tomato fruits are better preserved in refrigeration at 4 °C because the low temperatures maintain the quality characteristics of the tomato in postharvest.

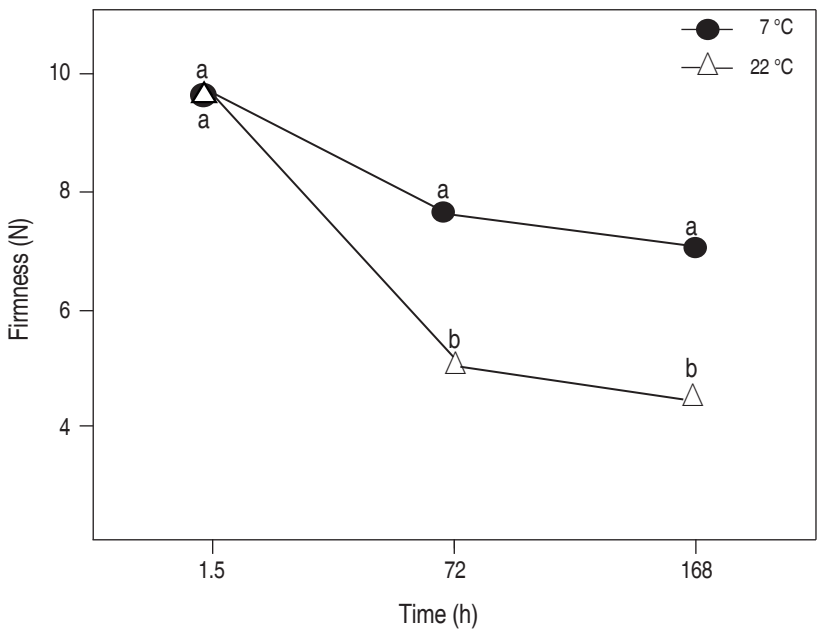

Figure 3. Firmness values of tomato fruits inoculated with *Escherichia coli* (ETEC) and stored at 7 and 22 °C. Different letters in each point time indicate a significant difference (Tukey, P<0.01).

Effect of storage conditions on organic acids and concentration of total soluble and reducing sugars

Citric acid is the most abundant acid in tomatoes and the largest contributor to titratable acidity. The decrease of acidity coincides with the increase in fruit ripening and is due to the loss of citric acid (Anthon *et al*., 2011). In this study, this parameter decreased significantly (P<0.05) as the ripening process advanced; the decrease was more notable in fruits stored at 22 °C (Table 1). In tomato, the ascorbic acid contents are higher in stage full maturation (De Oliveira *et al*., 2016), which is consistent with the results of this study that although no differences were detected in the three evaluated stages, the highest values were detected at 22 °C, where the fruit maturation occurs more quickly. While at 7 °C, a significant increase (P<0.05) was observed up to 168 h (Table 1).

Alenazi *et al*. (2020) pointed out that the content of total soluble sugars is related to tomato maturity. In this investigation, the total soluble sugars increased as the fruits ripened, and this was more accentuated in fruits stored at 22 °C than in those stored at 7 °C. In both conditions, significant differences were detected (P<0.05) at 72 and 168 h. According to Oms-Oliu *et al*. (2011), tomato fruit maturation involves changes in its physiology, for example, the increase in sugars, such as glucose and fructose, and sucrose reduction. This trend was more marked in the total soluble sugars in the fruits stored at 22 °C after 72 h.

The conservation of sugars in tomato fruits under refrigeration at 7 °C was not significantly different. This is similar to that reported by Buret *et al*. (1983), who confirmed that the sugar content in tomato fruits is constant at low

storage temperatures. At 22 °C fruits were significantly different in sugar content at some times, with an increase at 162 h. Fructose had statistically equal values in fruits stored at 7 and 22 °C, with a significant difference at the longest storage times (72 and 168 h). The fructose contents, associated with the decrease of some organic acids, contribute directly to the flavor of ripe tomatoes (De Oliveira *et al*., 2016). Finally, sucrose showed a slight increase after 168 h at 7 °C, while at 22 °C there was no significant difference with constant and minor values (Table 1). In this work, approximately equal amounts of the three sugars (glucose, fructose, and sucrose) were detected in the analyzed fruits regardless of the storage temperature.

CONCLUSIONS

Storage conditions significantly affected tomato fruits. ETEC survived with populations of 7 and 9.2 Log CFU g^{-1} at 7 and 22 °C, respectively, after 120 h of storage. The adherence and bacterial colonization in storage were confirmed by scanning electron microscopy. At 22 °C, glucose, sucrose, h^{o} parameters, and firmness were significantly more affected than at 7 °C. The concentration of fructose did not exhibit a significant difference at 7 °C.

The permanence of ETEC in tomatoes stored at 22 °C makes it necessary to propose strategies of sanitization process to minimize conditions of contamination and preserve the quality parameters of the tomato, during post-harvest storage to maintain the fruit's safety and prevent it from serving as a reservoir of enterobacteria capable of producing gastrointestinal disorders.

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Commercial meat cuts hardness of dual-purpose cattle from Cundinamarca (Colombia) high tropics zone

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Dureza de cortes comerciales de ganado bovino doble propósito del trópico alto en Cundinamarca, Colombia

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Keywords:
Breeding
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Shear force
Tenderness

ABSTRACT

This study aimed to determine the effect of biotype, slaughter age, sexual condition and feeding system on the four commercial meat cuts hardness: Lomo (*Longissimus dorsi*), Bota (*Biceps femoris*), Muchacho (*Semitendinosus*) and Paletero (*Infraspinatus*), from 50 bovines belonging to dual-purpose systems of the high tropics in Cundinamarca, Colombia. To obtain the values of this attribute, the Warner-Bratzler blade cutting force (F_{WB}) instrumental measurement methods and sensory evaluation were used, with 71% of correlation. This allowed the elaboration of a hardness scale in which the Lomo and Paletero meat cuts were in the category of "soft" and behaved statistically the same in both measurement methods, while Bota and Muchacho meat cuts were in the category of "medium" and had statistical differences according to the sensory panel perception. Likewise, the results showed significant differences between the meat cuts, highlighting Paletero due to lower hardness value and behavior similar to the Lomo meat cut. Significant effects on the meat toughness were found for all factors, except age, which shows the influence that these and their farm management have on the quality of the meat.

Palabras clave:
Crianza
Ganado doble propósito
Escala de dureza
Fuerza de corte
Terneza

RESUMEN

Este estudio tuvo como objetivo determinar el efecto del biotipo, la edad al sacrificio, la condición sexual y el sistema de alimentación sobre la dureza de cuatro cortes comerciales: Lomo (*Longissimus dorsi*), Bota (*Biceps femoris*), Muchacho (*Semitendinosus*) y Paletero (*Infraspinatus*), provenientes de 50 bovinos pertenecientes a sistemas de doble propósito del trópico alto en el departamento de Cundinamarca, Colombia. Para la obtención de los valores en este atributo se emplearon los métodos de medición instrumental de fuerza de corte con cuchilla de Warner-Bratzler y la evaluación sensorial, cuya correlación fue del 71%. Esto permitió la elaboración de una escala de dureza en la cual los cortes Lomo y Paletero se ubicaron en la categoría de "suave" (blando) y se comportaron estadísticamente igual en ambos métodos de medición, mientras que los cortes Bota y Muchacho se ubicaron en la categoría de "medio" y tuvieron diferencias estadísticas de acuerdo con la percepción dada por el panel sensorial. Así mismo, los resultados obtenidos mostraron diferencias significativas entre los cortes, destacando al Paletero por su menor valor de dureza y comportamiento similar al Lomo. Efectos significativos sobre la dureza de la carne fueron encontrados para todos los factores a excepción de la edad, lo cual muestra la influencia que tienen estos y su manejo en finca sobre la calidad de la carne.

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Meat and derived products are an important source of protein in human diets and their consumption depends on physiological factors (determined by individual characteristics), sensory (specific to the product), and the consumer environment. Among the sensory factors, tenderness and juiciness are quality attributes that positively influence consumer preferences and ensure acceptance, purchase, and loyalty to the product (Font-i-Furnols and Guerrero, 2014; Desgarennes *et al.,* 2017).

Tenderness is defined as the difficulty or easiness of cutting or chewing a piece of meat, being the result of factors intrinsic to the animal, the production system, and the post-mortem process. The first factors include sexual condition, age at slaughter, genetics, individual performance and nutrition, variables that can be managed and optimized within the production systems and affect the product obtained and its value in the market (Quiroga, 2005; Vásquez *et al.,* 2007; Braña *et al*., 2011; Torrico *et al.,* 2018).

Physicochemical properties including proximate composition, pH, color, water-holding capacity, tenderness, and sensory properties are different between meat cuts. Many studies have been conducted on meat quality traits of various meat cuts among the carcasses of different species (Cheng *et al*., 2020).

Tenderness has been quantified through instrumental methods; the Warner-Bratzler blade cutting force (Warner Bratzler shear force) is one of the most widely used tests due to its robustness and correlation with sensory measurement; likewise, the qualitative determination through a sensory panel is commonly used to determine the degree of tenderness or hardness of meat cut and their acceptance by consumers. In this way, international scales have been established that make it possible to correlate the values obtained by instrumental methods with sensory perception and thus classify various types of beef cuts (Destefanis *et al.,* 2008; Braña *et al*., 2011; Cuetia, 2012).

This study aimed to determine the influence of biotype, age at slaughter, sexual condition, and nutrition in dual-purpose animals in the high tropics on the hardness of four commercial meat cuts, through the instrumental measurement (Warner-Bratzler shear force test) and their relationship with to a sensory panel in order to create a scale to measure hardness locally.

MATERIALS AND METHODS

Experimental animal selection

In this study, 50 male bovines castrated (n=22) and non-castrated (n=28) with slaughter weights between 460 and 530 kg were selected from dual-purpose productive systems located in the highland tropics (above 1800 to 3500 masl) in Cundinamarca, Colombia. The animals studied were classified according to their biotype, considering the phenotype and the predominant characteristics in each one. The *taurus* biotype was composed of animals with characteristics of the Normande breed in the majority and small groups of Angus, Charolais, Simmental, Limousin and crosses with Normande. For the *indicus* biotype specimens of the Brahman breed were evaluated and for the cross biotype, animals of the Brahman x Normande, Charolais x Brahman, Simbrah and Brangus crosses were found. The ages at slaughter were classified into 2 ranges: from 22 months to 36 months for the group aged $\leq$ 3 years and animals from 37-38 months to 48 months of age at slaughter for the group >3 years. Age was determined according to farm records on the slaughter day or at the slaughterhouse through dental chronometry.

The diet of the animals was classified into 4 categories: stable, which was based on feeding *Pennisetum purpureum* or *Axonopus scoparius* grass, palm kernel cake, wheat, potato by-products and corn silage supplemented with the contribution of concentrate; rotational, which was described as grazing with a period of permanence in the pasture of 5 to 7 days and a rest period of the meadow that varied from 45 to 120 days depending on the type of grass (*Pennisetum clandestinum, Lolium perenne, Holcus lanatus, Paspalum dilatatum* and their associations), red clover (*Trifolium pratense*) and white clover (*Trifolium repens*); the third category was grazing in daily strips with rest days for the meadow that varied from 40 to 90 days depending on the used species (*Pennisetum clandestinum, Holcus lanatus* and their combinations), with red clover (*Trifolium pratense*) and white clover (*Trifolium repens*) in the grasslands. All systems were supplemented with mineral salt. The fourth feeding method was the "feedlot" which was characterized by the supply of vegetables, fruits, and tubers residues to the animals in confinement.

Meat cuts used

Four cuts of the bovine carcass were selected for the analysis: a high commercial value meat cut named in Colombia as Lomo (*Longissimus dorsi*), two meat cuts obtained from the hindquarter named in Colombia as Bota (*Biceps femoris*) and Muchacho (*Semitendinosus*), and a meat cut from front quarter carcass section named in Colombia as Paletero (*infraspinatus*). The results obtained for each meat cut were expressed in terms of hardness, i.e., resistance to cutting.

Sample handling

Once the carcasses were obtained in the slaughterhouse, they were transported to the processing plant and 3-4 kg of the commercial cuts were obtained. The meat cuts were transported to laboratory at the Food Science and Technology Institute (ICTA) of the Universidad Nacional de Colombia, Bogotá. Vacuum packages and refrigeration conditions (2.0±0.5 °C) were used for their preservation and further analysis.

Determination of instrumental hardness

The cuts (3-4 kg) were stored for 24 h in the meat and processed meats laboratory at ICTA at 2.0±0.5 °C. They were cut into steaks with a thickness of 3 to 4 cm and weights of 500±50 g. Then, the cuts were cooked on electric grill (Oster) until 71 °C; the method and cooking times followed the guidelines of the American Meat Science association (AMSA, 2015).

The obtention of samples was carried out based on the procedure proposed by Braña *et al.* (2011), in which the use of a meat cutter was specified to obtain cylinders (cores) with dimensions of 3x1x1 cm^3. The samples were placed on the base of the equipment in such a way that the muscle fibers were perpendicular to the Warner-Bratzler blade. The equipment used for these measurements was a TAXT plus electronic texturometer (Surrey, England) and associated software was Exponent 32 or TEE32 with the parameters of pre-test speed 1 mm s^{-1}, test speed of 2 mm s^{-1}, post-test speed of 10 mm s^{-1}, the force of the trigger of 0.005 kg and distance of 0.30 cm for the calibration of the equipment; curves were obtained by readings of the force exerted by the blade on the core, where maximum point represented the maximum cutting force or hardness.

Sensory evaluation

Sensory evaluation of 21 cuts from 10 animals selected within the group of 50 to be analyzed was carried out. The sensory panel consisted of 6 trained people who rated the samples in a format with linear scales from 0 as "very soft" to 10 as "very hard" in which the hardness attribute was presented. The training period of the sensory panel was 8 months at the Institute of Food Science and Technology (ICTA) in Bogota. It consisted of two different phases: the first one was focused on basic sensory training using the guides GTC 242 (2013) for color, NTC 3915 (2012) for taste, NTC 3929 (2009) for the flavor profile, NTC 4503 (2011) for detection of odors, and 4489 (1998) for food texture. The second phase consisted of the identification of sensory profile attributes in beef and the application of discriminative tests to different sensory traits which allowed panelists recognize and compare meat samples.

For the construction of specific hardness scale of the meat cuts used in the present work, various scales proposed by other authors, and used at the international level were consulted, such as methodologies proposed by Shackelford *et al.* (1995), Destefanis *et al.* (2008), and Calkins and Sullivan (2011) that allowed to structure the scale defining five ranges of hardness, to which a word was attributed, from very soft to very hard, based on the scale proposed for the rating of cuts by sensory panelists.

For the elaboration of the new scale, a regression equation was carried out from the data obtained by the sensory panel, which allowed calculating the shear force ranges corresponding to the scores given by the panelists on the scale.

Statistical analysis

A descriptive analysis of the data was carried out for each factor to be evaluated: type of cut, age at slaughter, sexual condition, biotype, and type of feeding. The effects of age at slaughter, sexual condition, biotype and type of feeding were analyzed using the *longissimus dorsi* values. Subsequently, the GLIMMIX procedure of SAS (Advanced Analytics Software, SAS Institute Inc, 2014) was used for the statistical analysis. This model allowed evaluating variables with a non-normal distribution using estimated means.

The response variable was the toughness of the meat obtained by the instrumental method, the farm was considered as a random effect and the other variables as fixed effects. Analyzes were performed to determine the presence of significant differences for each of the fixed factors through the type III fixed-effects test. After determining the presence of significant differences with $P<0.05$, the Tukey-Kramer multiple range test was used.

For sensory analysis, the IBM® SPSS Statistics® version 17 software was used, performing descriptive statistics and non-parametric statistics. The Kruskal-Wallis test was used to verify the presence of significant differences between the cuts and the Bonferroni adjustment and Dunn's test of multiple comparisons by pairs were carried out to create the groups.

The Spearman correlation coefficient was found for the variable of sensory hardness and instrumental hardness, which allowed establishing a relationship between these two parameters.

RESULTS AND DISCUSSION

Hardness according to the type of meat cut

The results obtained are presented in Table 1. Significant differences were found between the cuts for the instrumental hardness variable expressed in kgf ($P<0.0001$). The meat cuts Lomo and Paletero behaved statistically the same, being Paletero the lowest value with 5.794 kgf followed by Lomo with 5.988 kgf. The meat cuts Bota and Muchacho were statistically equal and presented the values of 7.781 kgf and 8.097 kgf, respectively, the latter being the highest value.

Muscles are classified as postural or locomotor depending on their metabolic and contractile functions; they differ from each other by the composition and size of their muscle fibers, as well as the presence of connective tissue (Bruce and Alhus, 2017). In this way, the results are consistent with those reported by Belew *et al.* (2003) who found lower hardness values in postural-type muscles (*Longissimus dorsi, Infraspinatus*) when compared with locomotor-type muscles (*Biceps femoris, Semitendinosus*). This is explained by the greater presence of connective tissue in the locomotion muscles due to their high degree of utilization and the hypertrophy of myofibers due to exercise. Calkins and Sullivan (2011) and Guo and Greases (2017) stated that collagen is not evenly distributed in the different skeletal muscles; those located in the back region of the animal have less connective tissue than the muscles of the thoracic region and these, in turn, present less than the muscles of the pelvic region. Likewise, muscles with shorter fibers tend to be tenderer than those with longer muscle fibers (Pinilla, 2014; Bruce and Alhus, 2017).

Table 1. Hardness according to the type of meat cut.

Quality trait	Meat Cut	
	Common name in Colombia	Estimated Mean ± SE*
Hardness (kgf)	Lomo (*Longissimus dorsi*)	5.988±0.273 a
	Bota (*Biceps femoris*)	7.781±0.273 b
	Muchacho (*Semitendinosus*)	8.097±0.273 b
	Paletero (*Infraspinatus*)	5.794±0.273 a

* Different letters (a, b) show significant differences ($P<0.05$). kgf: kilograms force, according to the Technical System of Units.

Sensory analysis

Table 2 shows the differences found for the four cuts evaluated by a sensory panel and the Warner-Bratzler blade shear force method (F_{WB}). The meat cut Paletero was considered the softest meat cut in both cases, followed by the meat cut Lomo; the panelists considered them statistically equal, which was consistent with the results found with the F_{WB} method. The meat cut Bota presented

lower values of cutting force than the meat cut Muchacho, but statistically, they behaved the same for this method; however, the meat cut Muchacho was considered by the panelists as softer than the meat cut Bota and presented statistical differences.

A hardness scale was proposed that allowed classifying the meat cuts according to the panelist's perception. This scale was established once the Spearman correlation coefficient between the Warner-Bratzler blade shear force (F_{WB}) test (instrumental hardness) and the sensory panel (sensory hardness) was calculated, which was 71% (Figure 1). Calkins and Sullivan (2011) found a Pearson correlation coefficient of -0.84 (P<0.01) between sensory measured tenderness and instrumental hardness. On the other hand, Destefanis *et al.* (2008) found a Pearson correlation coefficient of -0.72 (P<0.01) for the same variables. The negative values are explained by the fact that scales in the mentioned works started from 1 as the value of greatest hardness.

Table 2. Variations in sensory and instrumental hardness for the meat cuts evaluated.

Meat Cut	N*	Hardness determined by the sensory panel					Hardness determined by the Warner-Bratzler blade cutting method (kgf)
		mean**	SD	VC	Min	Max	Estimated Mean ± SE**
Lomo	7	4.41 a	1.79	0.41	1	7	5.988±0.273 a
Bota	5	6.88 c	1.13	0.16	4	9	7.781±0.273 b
Muchacho	5	6.19 b	1.51	0.24	3	9	8.097±0.273 b
Paletero	4	4.07 a	1.49	0.37	1	7	5.794±0.273 a

*Number of samples. **Values in the same column with different letters (a, b, c) show significant differences (P<0.05).
kgf: kilograms force according to the Technical System of Units. SD: Standard deviation. VC: Variation coefficient

The regression equation of the measurements of Warner-Bratzler shear force (F_{WB}) test and the values obtained in the sensory panel was created to calculate the cutting force value corresponding to the scores given by the panelists on the scale: y = 0.6103x + 3.7641; where: y = Instrumental hardness and x = Sensory hardness.

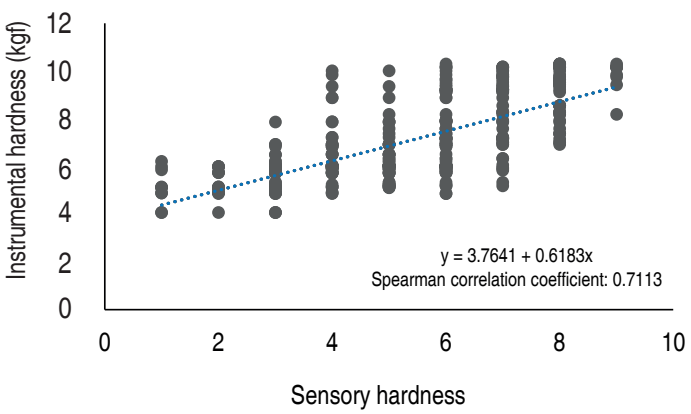

Figure 1. Relationship between instrumental and sensory hardness (hardness values obtained for 21 meat cuts evaluated using the Warner-Bratzler blade cutting force (FWB) as instrumental method and the sensory panel).

The values obtained from the sensory panel scale were compared with the results obtained by the instrumental test (F_{WB}) for the same meat cuts, thus, the hardness scale could be generated for the meat evaluated in this experiment.

According to the scale of hardness proposed for this study (Table 3), the meat cuts Lomo and Paletero are considered within the category of "soft" while the meat cuts Bota and Muchacho are classified within the "medium" category, although it should be mentioned that the meat cut Muchacho had similar values to the lower limit of the "hard" category (it presented a higher hardness value (F_{WB})) than meat cut Bota. The largest differences with the scales found in the literature (Table 4) were in the Warner-Bratzler blade shear force (F_{WB}) values that defined the limits for each category; for example, the lower limit of the proposed scale is < 4.380 kgf with a sensory interpretation of "very soft", while in the scales used as a reference, the trait "very soft" or "soft" were assigned to values less than 3.9 kgf.

Table 3. Hardness scale proposed for meat cuts in this study *.

Category	Range (kgf)	Sensory interpretation
1	<4.380	very soft
2	4.380-6.240	soft
3	6.241-8.100	medium
4	8.101-9.950	hard
5	>9.950	very hard

kgf: kilograms force according to the Technical System of Units. *The category was determined according to the equivalence in instrumental hardness values by the F_{WB} method and its sensory interpretation.

The shear force values (F_{WB}) for the four meat cuts evaluated in this study are considered in the category of "extremely hard" according to the scale of Shackelford *et al.* (1995); the meat cuts Lomo and Paletero were located in the category of "hard" according to the scale of Destefanis *et al.* (2008) and Calkins and Sullivan (2011), while the meat cuts Bota and Muchacho were considered "very hard " according to Destefanis *et al.* (2008) and "hard" as the upper limit for Calkins and Sullivan (2011). According to the classification given by those authors, the meat cut Paletero (IF) was considered one of the softest meat cuts with average values of 3.2 kgf and sensory category "soft" as in this research. The meat cut Lomo presented values that ranged between 4.07 and 4.20 kgf, which means that it belongs to the intermediate sensory category in the scales used as reference (Table 4), while was considered soft in the scale proposed in the present work.

Table 4. Bovine meat cuts toughness classification scales of different authors.

Category A	Sensory interpretation	Mean $F_{(WB)}$ (kgf)	Category B	Sensory interpretation	Mean $F_{(WB)}$ (kgf)	Category C	Sensory interpretation	Mean $F_{(WB)}$ (kgf)
1	Very Soft	>6.38						
2	Soft	5.38–6.38	1	Tender	<3.9	1	Tender	<2.27
3	Medium	4.37–5.37	2	Medium	3.9–4.6	2	Moderately tender	2.27–3.63
4	Hard	4.36–3.36	3	Hard	>4.6	3	Hard	3.63–5.44
5	Very Hard	<3.36				4	Extremely hard	>5.44

Category A: scale proposed by Destefanis *et al.*, 2008. Category B: scale created by Calkins and Sullivan, 2011. Category C: scale proposed by Shackelford *et al.*, 1995. kgf: kilograms of force, according to the Technical System of Units.

Feeding systems

The four feeding systems evaluated were rotational grazing and strips grazing, whose diet was based on forages supplied through grazing, the stable system

with a diet of grains and cut forages, and feedlot with vegetable residues. Significant differences (*P*=0.0007) were found between feeding systems for instrumental hardness.

The shear force values obtained for the forage-based diets, both in rotational grazing and strips (did not show significant differences between them) were the lowest in comparison with the other diets and feeding systems (Table 5). The group of stabled animals did not show significant differences with the grazing groups, which agrees with that reported by Lage *et al.* (2012) in the tenderness of loins from animals supplemented with concentrate at 0.8 and 1.2% of body weight. In contrast, Jiang *et al.* (2010) found no significant differences in shear forces and sensory perceptions of the tenderness of loins from animals in a feedlot system (finishing with alfalfa and grains), one diet with triticale and *ryegrass*, another one based on triticale forage and kale, and the third one with *ryegrass*, fescue and finished in a feedlot of alfalfa and grain; although Jiang *et al.* (2010) reported that significant differences were found in juiciness and flavor due to lower marbling of the loins from forage-based diets.

Table 5. Hardness according to the feeding method.

Quality trait	Feeding method	
	Category	Estimated Mean ± SE*
Hardness (kgf)	Strips	6.592±0.282 a
	Rotational	6.590±0.216 a
	Stables	7.196±0.473 ab
	Feedlot	8.348±0.399 b

*Different letters (a, b) show significant differences (*P*<0.05). kgf: kilograms force, according to the Technical System of Units.

Likewise, it was found that the group of stabled animals had higher values of shear force (hardness) than grazing animals, in contrast to what was found by other authors who have reported that animals fed with pasture-based diets showed higher values of shear force with a Warner-Bratzler blade and presented the worst scores in tenderness given by consumers, compared to those fed with high protein and energy diets. This was attributed to the low percentage of marbling in the carcasses found in those studies (Warner *et al*., 2010; Sterman and De Felício, 2010).

Camacho (2008) pointed out that high protein and energy diets, typically those with a high percentage of concentrate or grains, especially in the finishing phase, lead to obtaining heavier carcasses with higher yields, due to rates of higher growth rates that allow reaching slaughter weights in a shorter time. Higher levels of marbling or intramuscular fat deposition and greater fattening of the carcass have also been found, while meats produced based on forages presented less marbling, darker colors, and higher hardness values (Vásquez *et al.,* 2007). Batista *et al.* (2016) demonstrated this assertion by finding that finishing diets rich in energy increased the deposition of adipose tissue in animals of the Nellore breed and had a positive impact on the tenderness of the meat.

On the other hand, the last group evaluated in this study belonging to a feedlot system behaved statistically different from the group of grazing animals but had a statistically equal behavior to the housed group presenting higher hardness values. These results can be attributed to the content of the diets supplied and their nutritional value, since the diet was based on residues of vegetables, fruits, and tubers, with a lower energy content than that found in commercial concentrate.

Sexual condition

This factor significantly affects intramuscular fat deposition, juiciness, and palatability in meat (Vásquez *et al*., 2007). The results for instrumental hardness obtained through the Warner-Bratzler blade shear force (F_{WB}) test for Lomo (*Longissimus dorsi)* are presented in Table 6. The loin cut was chosen because of its shear force and sensory values, also, this muscle is widely used in meat science studies to report quality traits. Significant differences were obtained between the two categories analyzed, the group of castrated animals was the one that presented the lowest hardness values (6.512 kgf) when compared with the non-castrated group, which had an average value of 7.476 kgf. This contrasts with the results found by Miguel *et al.* (2014) who did not find significant differences in the hardness values of late immunocastrated animals, non-castrated, and surgically castrated males.

Table 6. Hardness according to sexual condition.

Quality trait	Sexual condition	
	Category	Estimated Mean ± SE*
Hardness (kgf)	Castrated	6.512±0.195 a
	Non-castrated	7.476±0.239 b

* Different letters (a, b) show significant differences (*P*<0.05). kgf: kilograms force, according to the Technical System of Units.

Vásquez *et al.* (2007) and Latorre *et al.* (2017) found lower hardness values in Lomo cuts from castrated animals (4.50 to 4.75 kgf) when compared with non-castrated animals of the same age (4.9 at 5.0 kgf) but it did not show significant differences. It was also found that at the sensory level, the meat of non-castrated adult males was rated as less tender compared to the meat of castrated animals or heifers of the same age (Vásquez *et al.*, 2007). Panjono *et al.* (2009) and Amatayakul *et al.* (2012) determined that the lower hardness values in castrated animals were attributed to their greater degree of fattening and marbling and their lower rate of maturity at slaughter, which according to the authors, was caused by the effect testosterone has on the animal by generating changes in the connective tissue through the formation of insoluble cross-links and a lower deposition of intramuscular fat, aspects that could influence the results obtained in this study.

Age at slaughter

The results obtained for instrumental hardness with the age at slaughter are presented in Table 7. Although various authors have reported statistically significant differences for the hardness values in the meat from animals of different ages, the results obtained in this work show that there were no significant differences between the age groups young (than or equal to 3 years) and old (than 3 years). Even so, it is evident that the animals within the young group presented lower hardness values (6.742 kgf) than those found in the old group (7.391 kgf).

Torregroza *et al.* (2016) and Vásquez *et al.* (2007) found that animals with a high growth rate presented a higher content of type III collagen, which is characterized by being halo-soluble and having less complexity in its structure and concluded that the meat of adult cattle is harder than young cattle, a fact that is evidenced in the results of this study. Camacho (2008) reported that in addition to the connective tissue content, the increase in the diameter of the muscle fibers given after 30 months of age generates greater resistance to cutting, especially in grazing animals such as those in this study. In this way, the higher hardness values can be explained in animals whose age range is greater than 3 years.

Table 7. Hardness according to age at slaughter.

Quality trait	Age at slaughter	
	Age group*	Estimated Mean ± SE**
Hardness (kgf)	≤ 3 years	6.742±0.176 a
	> 3 years	7.391±0.314 a

* Group ≤3 years old made up of animals from 22 to 36 months of age at slaughter. Group >3 years old made up of animals from 37 to 48 months of age at slaughter. ** Different letters (a, b) show significant differences (*P*<0.05).

Biotype

The results obtained for hardness according to the animal biotype are presented in Table 8, where the presence of statistical differences was found (*P*=0.0053). The hardness values found for the *Bos indicus* animals (average of 8.265 gf) were statistically different from the other groups of animals that obtained lower values, which agrees with that reported by Hernández (2008) who stated that the meat of animals Cebu type presents weaknesses in some palatability characteristics such as hardness. This can be attributed to the lower post-mortem proteolysis resulting from the high activity of Calpastatin on Calpains that hydrolyze the contiguous bonds to cysteine (especially CAPN-1), playing a very important role in the degradation of post-mortem muscle fibers, and the maturation process (Peluffo and Monteiro, 2002; Pinilla, 2014; Desgarennes *et al.*, 2017; Wright *et al.*, 2018). It has also been reported in the literature that the degree of crossbreeding of animals generates differences in the tenderness of the meat, specifying that 25% *Bos indicus* breed in the animal already generates variation in the tenderness of the cuts; that is why meats from animals with 50% Brahman breed are less tender than meats from animals with 25% Brahman breed; which makes sense when evaluating Calpastatin

increasing activity and the decreasing in post-mortem proteolysis (Camacho, 2008; Montoya, 2014; Wright *et al*., 2018).

Table 8. Hardness according to the biotype of the animals.

Quality trait	Biotype	
	Group according to biotype	Estimated mean*
Hardness (gf)	*Bos indicus*	8.265±0.360 a
	Bos taurus	7.093±0.208 b
	Cross breeding	6.284±0.289 b

*Different letters (a, b) show significant differences (P<0.05). kgf: kilograms force according to the Technical System of Units.

The lowest hardness value in this experiment corresponded to the biotype cross-breeding with 6.284 kgf made up of individuals from the Brahman x Normande, Charolais x Brahman, Simbrah and Brangus cross-breeding. The *Bos taurus* biotype composed mostly of animals of the Normande breed and their crosses with meat breeds presented values of 7.093 kgf.

The type of cross-breeding also affects the level of tenderness in the meat cuts. Hernández (2008) found that there was a lower age at slaughter in the cross-breeding of Sanmartinero×Cebu and Simmental×Cebu compared to the Cebu pure breeding and cross-breeding with Romosinuano, breeds with rapid growth allow the animals to be slaughtered at lower ages, obtaining better tenderness values (lower F_{WB}). In this study, better tenderness values were obtained in the meat cuts from cross-biotype breeding concerning *Bos taurus* and *Bos indicus*. It is important to note that the animals of the *Bos taurus* group in this work were mostly Normande specimens and cross-breeding with Normande, breeds that are not specialized in meat production and do not show rapid growth, that is the reason why values were attributed to superior hardness to this biotype when compared with the cross-breeding group.

Vásquez *et al.* (2006; 2007) reported shear force values greater than 5 kgf in loins from Brahman and Brangus breeds animals when compared with pure Romosinuano, Romosinuano×Cebu and Cebu×*taurus* mixtures, obtaining values of 4.23, 4.78 and 4.26 kgf, respectively; this trend was also presented in the results obtained for this work, where the values of shear force obtained for cross-bred animals were found to be smaller than pure breeds, and the highest values were found in *Bos indicus* biotypes. Results for hardness when comparing biotypes agree with values reported by Lage *et al.* (2012) who compared the meat cuts of Nellore breed animals with Angus and Simmental×Nellore, always obtaining the highest hardness values in Nellore.

CONCLUSIONS

The instrumental and sensory hardness of the meat cuts are largely determined by the handling given to the animals within the production systems. Likewise, the type of muscle used has a direct impact on the final hardness perceived by the consumer due to the nature of the muscle fibers, their level of development and their anatomical location. In this way, a potential use of the Paletero meat cut was found due to its similarity to the Lomo. The high correlation was obtained between the instrumental method and the sensory method allowed the creation of a hardness scale for the classification of meat cuts and represented the first approach made for meat cuts in the region of Cundinamarca, Colombia. Its application is suggested in areas with similar characteristics to those found in this project.

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Optimization of the extraction and precipitation process of a leaf protein concentrate from *Moringa oleifera* Lam.

UNIVERSIDAD NACIONAL DE COLOMBIA

Optimización del proceso de extracción y precipitación de un concentrado proteico foliar de *Moringa oleifera* Lam.

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Keywords:
Isoelectric precipitation
Moringa oleifera Lam.
pH solubilization
Plant protein.

ABSTRACT

This study aimed to determine the best extraction and precipitation conditions of *Moringa oleifera* Lam. leaf protein. The influence of pH (10, 11, 12) and the concentration of NaCl (0, 0.25, 0.5) for the protein extraction process were studied through a Completely Randomized Design (CRD) with factorial arrange 3^2. The combination of pH 11 and 12 with 0 M NaCl had the best yield (P<0.05). The treatment of pH 11 without NaCl followed a precipitation stage for its purification, and the effect of different levels of pH (4, 4.5, 5) and temperature (40, 60, 80 °C) were evaluated using a CRD with factorial arrange 2^2 and 6 central points. The temperature did not affect the yield of the process in a significant way and the amount of precipitate was maximized at pH 4 and 4.5. From 100 g of the dry leaf, 7.26±0.19 g of protein was isolated with a recovery of 26.93±0.22 g 100 g^{-1} from the total protein. Due to their astringency and bitterness, consuming large amounts of *Moringa oleifera* Lam leaves is not a solution; therefore, obtaining a leaf protein concentrate could be useful for diverse applications in nutritional supplements, and as raw material for functional products development.

Palabras clave:
Precipitación isoeléctrica
Moringa oleifera Lam.
Solubilización por pH
Proteína vegetal

RESUMEN

El objetivo de este estudio fue determinar las mejores condiciones de extracción y precipitación de la proteína foliar de *Moringa oleifera* Lam. Se estudió la influencia del pH (10, 11, 12) y concentración de NaCl (0, 0,25, 0,5) en el proceso de extracción de la proteína de *Moringa oleifera* Lam a través de un Diseño Completamente al Azar (DCA) con arreglo factorial 3^2. La combinación de pH 11 y pH 12, ambos sin NaCl, presentaron el mayor rendimiento (P<0.05). El tratamiento a pH 11 sin NaCl continuó la etapa de precipitación para su purificación, evaluando el efecto de diferentes niveles de pH (4, 4,5, 5) y temperatura (40, 60, 80 °C) utilizando un DCA con arreglo factorial 2^2 con 6 puntos centrales. La temperatura no afectó significativamente el rendimiento del proceso y a pH 4 y 4.5 se maximizó la cantidad de precipitado obtenido. A partir de 100 g de hoja, se aislaron 7,26±0,19 g de proteína con una recuperación de 26,93±0,23 g 100 g^{-1} de la proteína total. No es posible consumir las hojas de *Moringa oleifera* Lam en grandes cantidades debido a su astringencia y amargor, por lo que el obtener un concentrado proteico foliar podría ser útil en diversas aplicaciones como suplementos nutricionales y materia prima para el desarrollo de alimentos funcionales.

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Moringa oleifera Lamarck (MO) belongs to the Moringaceae family. Although this plant is originally from India, it is well distributed around the tropical zones of the planet. It is wildly recognized as a drought-resistant species with many applications in animal and human nutrition. MO can grow in many different environmental conditions and it has been reported to produce up to 580 t ha^{-1} per year of fresh sprouts. For these reasons, The Food and Agriculture Organization of the United Nations (FAO) recommends Moringa as a potential crop (FAO, 2019). There are no official reports of its growth in Ecuador, however, there are private initiatives such as Ecuamoringa, which manages a wide national market.

MO leaves have important nutritive qualities among perennial vegetables including their protein content between 24-29 g 100 g^{-1}, and important amounts of vitamins A and C, and minerals such as Ca, Fe and P. MO protein contains all the essential amino acids in important concentrations and a good amino acid profile reaching 72.4% (Alain *et al*., 2016), despite the high amount of leaf required to obtain them, and the low protein digestibility-corrected amino acid score (PDCAAS) 41.42% related to antinutritional factors in the plant. Amino acids are essential for the human body, being used as synthesis precursors of proteins, peptides and low molecular weight molecules. The absence of these compounds causes sickness as kwashiorkor and marasmus, which are common in developing countries (Wu, 2016).

Finding new protein sources is a key strategy to face the fast population growth and the environmental problems of big-scale cattle growing. Although in developed countries animal meat and its derivatives are still the main sources of protein, investigation of alternative protein sources is growing in popularity (Haque and Varshney, 2015).

Peptides of MO have demonstrated antioxidant capacity and the ability to inhibit the angiotensin-converting enzyme (ACE). These effects depend on the amino acid type and composition of the peptides formed in the stomach through the synergism of internal peptide enzymes (Saucedo-Pompa *et al*., 2018).

Moringa has applications in traditional medicine to treat diabetes. This quality has been principally attributed to its secondary metabolites. Moreover, different studies have demonstrated the pharmacologic properties of the foliar protein of different vegetables. Specifically, Paula *et al*. (2017) have proved the possibility to reduce the blood glucose level up to 66.4% by administrating 500 mg kg^{-1} of an aqueous extract of MO leaves; as well as the significant reduction off malonaldehyde (MDA) synthesized by the body in the presence of high concentrations of reactive oxygen species (ROS), which cause oxidative stress and mutagenic problems (Nishikawa *et al*., 2000).

Despite the health benefits obtained from MO consumption, direct consumption of high amounts of vegetable leaves could cause different disadvantages, especially in nutritional terms due to their antinutritional components and fiber. Neither trypsin nor amylase inhibitors have been detected in MO leaves, but important concentrations of phenols, tannins, saponins and phytates decrease nutrients' bioavailability in this crop (Makkar and Becker, 1997).

Furthermore, MO has a bitter and astringent flavor caused by flavonoids such as catechins and glucosinolates, specifically 4-(rhamnopiranosiloxi) benzil glucosinolate and monoacetil (rhamnopiranosiloxi) benzyl glucosinolate; this latter group is transformed to isothiocyanates by the myrosinase enzyme in the mastication process, incrementing the pungent and spicy flavor, so food likeness decreases (Doerr *et al*., 2009). In this context, it is valuable to isolate a leaf protein to use it as a supplement or raw material in the development of nutritious food products and further pharmacological applications.

Solubilization of protein is the first step in isolating protein from other leaf compounds. There are many different methods to separate protein such as the use of organic solvents, aqueous extraction and enzymes. The most used method is the aqueous extraction with addition of salts or changing the pH of the solvent, due to its low cost (Tan *et al*., 2011). Alkaline extraction based on Osborne (1924) protocol is commonly used in the industry, and the addition of different salts of Na^{+} and Ca^{+} has been reported beneficial for protein extraction in some food materials (Martinez-Maqueda *et al*., 2013). There is not much information about the interaction of salt concentration and alkaline pH in vegetable protein extraction and solubilization.

MO has all the protein fractions described by Osborne and Voogt (1978): water-soluble albumins, saline soluble globulins, 70-90% ethanol-soluble prolamins and acid alkali-soluble glutelins (Teixeira *et al*., 2014), which means that the alkaline and salt extraction could be used in order to obtain more protein while avoiding organic solvents such as ethanol because of the high economic and environmental costs. Furthermore, the leaf protein must be separated from the other compounds in the extracted solution. A good strategy is the use of isoelectric precipitation, as well as the use of temperature to reduce protein solubility. As a strategy to deal with the rapid population growth and the environmental problems generated by large-scale livestock farming, it is important to seek new sources of protein. Therefore, this study aimed to optimize the conditions of the *Moringa oleifera* Lam. leaf protein extraction and precipitation processes, as a green and eco-innovative alternative to obtain protein, concentrates with nutritional quality by reducing the effect of other components present in the matrix food and that can compromise their quality.

MATERIALS AND METHODS

Raw material

The leaves of *Moringa oleifera* Lam. were obtained from Ecuamoringa S.A., an Ecuadorian company, headquartered in Guayaquil that operates mainly in the agricultural area and stands out as a producer of MO. The drying process was performed at 35 °C until obtaining constant weight. The material was grounded and standardized using a mesh sieve of 0.25 mm (Teixeira *et al*., 2014).

Reagents

Hydrochloric acid (PubChem CID: 313; 37%, MERCK); Sodium hydroxide (PubChem CID: 14798, Fisher Scientific); Kjeldahl catalyzer (Cu-Se), (Scharlau); sulfuric acid (PubChem CID: 1118, MERCK; Antifoam (Sodium sulfate 97% and silicone 3%, Velp Scientific); Boric acid (PubChem CID: 7628, Loba Chemie); Bradford reagent and Bovine Serum Albumin (BSA) (PubChem CID: 16132389, Sigma-Aldrich). These reagents were imported from the United States (Fisher Scientific, Merck, Sigma-Aldrich and Velp Scientific), Spain (Scharlau) and India (Loba Chemie).

Protein extraction process

The methodology of Tan *et al*. (2011) was followed with modifications: 20±0.1 g of milled leaf were weighted with an electronic analytical balance (ML204, Mettler Toledo, Zurich Switzerland) with a deviation of ±0.1 mg scale in a 500 mL Erlenmeyer flask to finally add the water-NaCl solution in relation 1:10 w/v. The sample was homogenized for 5 min in VELP Scientifica stirring plate at 800 rpm. The pH was adjusted to 10, 11 and 12 with NaOH 1 N and measured with a Mettler Toledo model Seven Compact potentiometer according to the experimental design. The Erlenmeyer was placed in a shaking bath (Julabo SW22) at 25 °C at 200 rpm for 1 h. The pH was rectified each 10 min.

Experimental design

A Complete Randomized Design (CRD) was used with a factorial arrange of 3^2, resulting in the combination of pH (10, 11 y 12) and NaCl concentration (0, 0.25 and 0.5 M). The nine treatments E1 (pH 10, 0 M); E2 (pH 10, 0.25 M); E3 (pH 10, 0.5 M); E4 (pH 11, 0 M); E5 (pH 11, 0.25 M); E6 (pH 11, 0.5 M); E7 (pH 12, 0 M); E8 (pH 12, 0.25 M); E9 (pH 12, 0.5 M) were performed in triplicate obtaining 27 experimental units. The response variable was the protein extraction yield.

Protein analysis

Kjeldahl method .The total content of protein in the extract was determined by the Kjeldahl method (AOAC 991.22) (AOAC, 2019). The conversion factor used was 6.25 (Mbailao *et al*., 2014). Three temperature ramps were programed (140 °C for 15 min, 250 °C for 20 min and 420 °C for 40 min) in the protein VELP Scientific DK 6 digester to avoid overboiling of the sample. The distillation was performed in a VELP Scientific UDK 132 distillatory.

Bradford method. To use a less expensive and faster method for the quantification of protein in the extract, the Bradford method was investigated and compared with the Kjeldahl method. A Bradford test kit was used for this purpose (Sigma Aldrich). A calibration curve was constructed using BSA as standard. For it, 5 uL of the sample were mixed with 250 uL of the Bradford reactive and were incubated in a dark camera for 25 min. To measure the optical density inside the range of the calibration curve, each one of the protein samples was diluted in a ratio of 1/15, 1/20 and 1/25 with pure water. The optical density was measured in an Elisa MRX Microplate Reader from Dynex Technologies (Denkendorf, Germany) at 595 nm.

Protein extraction and precipitation yield

The comparison between the extracted protein and the original amount of protein in the leaf previous to the solubilization process was used to calculate the protein extraction yield. Also, the protein precipitation yield was calculated by comparing the remaining protein from the supernatant with the original protein concentration in the extract.

Relationship between protein analysis methods

The Pearson correlation coefficient determined the relationship between both methods. The Tukey test was used to compare the results of each treatment by different methods of analysis.

Precipitation process

The best treatment from the protein extraction process continued with the precipitation stage following the methodology of Serpa-Guerra *et al.* (2014) with modifications. From the extract, 1300 mL were divided into 100 mL aliquots. The pH was adjusted with a solution of HCl 1N following the experimental design and the temperature of the shaking bath configured to 100 rpm for 30 min.

The samples were put in 4 Falcon tubes of 50 mL to be centrifuged (Hermle Z206A) for 10 min at 5380 rpm to separate the supernatant from the protein (precipitate).

Experimental design

The treatments were analyzed by a CRD with factorial arrange 2^2 with the factors pH (4 and 5) and temperature (40 and 80 °C). Each treatment was performed twice and 6 central points were added to obtain 5 treatments: P1 (pH 4, 40 °C); P2 (pH 4, 80 °C); P3 (pH 5, 40 °C); P4 (pH 5, 80 °C); P5 (pH 4.5, 60 °C). The precipitation yield was used as a response variable.

RESULTS AND DISCUSSION

Protein extraction

The purpose of the first stage of this study was to determine the best combination of pH and NaCl concentration to obtain the maximum extraction yield from MO leaves. The original content of protein from the leaf was 26.96±0.24 g 100 g^{-1}. Similar results were obtained by Olson *et al.* (2016) (27.3 g 100 g^{-1}). This value was used to calculate the protein extraction yield. The calibration curve for the Bradford methodology had the regression equation y=0.2927X+0.4408 with a determination coefficient (R^2) of 0.9954.

A significant difference was found between the treatments (P<0.05), as well as in the influence on the protein content of the pH, NaCl factors and their interaction by both analysis methods (Table 1).

Table 1: Summary of the Analysis of Variance (ANOVA) of protein extraction yields.

Sources of variation	DF	Mean Squares	
		Kjeldahl method	Bradford method
Total	26		
Treatments	8	92.18 *	222.32 *
Salt (A)	2	295.01 *	432.54 *
pH (B)	2	46.69 *	407.34 *
Interaction A x B	4	13.09 *	24.70 *
Residue	18	1.03	1.85

*Significant at 5% of probability by the F test. DF: degrees of freedom

According to Condo and Pazmiño (2015), the coefficient of variation (CV) in experiments carried out in the laboratory should be up to 5%, to show the reliability of the research. In the present study, both Kjeldahl and Bradford methods presented a CV of 3.23 and 3.9% respectively.

The medium pH is the decisive factor in the solubility of the protein. The net charge of the protein in certain pH depends on the pKa values of the ionized groups of the protein. There are three different possibilities for the isoelectric point (pI): the net charge is 0, the net charge is positive in pH values lower than the pI and values higher than pI generate negative charge. The solubility of the molecule depends on the molecule charge. When pH is equal to pI, the solubility is minimum, but in higher and lower pH values, the solubility forms a “U shape”

distribution with higher solubility in alkaline pH (Zayas, 2012). In addition to the increase in protein solubility, the alkali hydrolyzes the protein bounded with polyphenols and polysaccharides decreasing their hydrophobic characteristic. Furthermore, the cellular wall is damaged by the NaOH creating cracks and increasing the diffusion rate (Zhang *et al*., 2015).

On the other hand, MO leaves have an important content of phytates that could be higher than in other legumes. Phytates bind to certain minerals such as Ca^{+} or Mg^{+}, and proteins, forming insoluble complexes that reduce bioavailability. This effect could also be influenced by the inhibition of digestive enzymes entrapping them or chelation of their principal substrate in the organism: calcium. Through the extraction process in an alkaline medium, the pH can alter the phytate-protein complex and with sufficient amounts of Ca^{+} (present in high concentrations in MO), these molecules will precipitate and could be separated from the extract before the precipitation of proteins (Rham and Jost, 1979).

In addition, the saline concentration of the medium influences the ionic strength; however, the mechanism by which it influences the protein solubility is still unknown (Zayas, 2012). Salts interact with protein-charged groups decreasing the electrostatic attraction and improving the relationship between the molecule and the solvent. When the saline concentration reaches a determinant point, the water molecules are not able to support the burden of the ions and proteins, so the less soluble solute is precipitated (protein) (McQuarrie and Simon, 1997). Since the charged groups of protein that interact with the ions of the salts depend on the pH of the medium, the effect of the saline concentration is related to the pH as could be seen by the significant interaction between both factors (P<0.05) (Table 1).

Both treatments E4 (pH 11) and E7 (pH 12) without salt were statistically equal between them. The extraction yields were 40.4 and 38.79 g 100 g^{-1} by the Kjeldahl method and 38.11 and 40.28 g 100 g^{-1} by the Bradford method, respectively (Table 2). These results are similar to those reported by Zhang *et al.* (2015) in alkaline extraction from tea leaves (*Camellia sinensis)* and 41.5 g 100 g^{-1} obtained by Coldebella *et al.* (2013) in *Manihot esculenta* leaves with a similar methodology.

The protein extraction yield related to the increase in pH value is explained by the increase of the negatively charged groups of the protein that generate electrostatic forces, which avoid molecules from joining and precipitating (Hou *et al*., 2017).

Similarly, salts in low concentrations generally improve the solubility of the protein, stabilizing it through non-specific electrostatic interactions (Perez-Jimenez *et al*., 2004). Nevertheless, in the conditions of this experiment with high charged proteins due to alkaline pH, the salt arouses the electrostatic repulsion by masking the charges and reducing the solubility in consequence (Dahal and Schmit, 2018).

The Bradford method is used to analyze protein content as an alternative to the Kjeldahl method. It uses Coomassie Brilliant Blue (CBB) as the colorant, which creates a complex with protein by electrostatic interaction and Van der Waal forces. This method is especially used to measure soluble protein because it is fast and cost-efficient (Palada *et al*., 2007). It measures the increase in absorbance of the sample after the addition of the dye and the incubation for a specific time, but if the time exceeds, precipitation of the complex is possible.

The complex is insoluble from the beginning of its formation, and the accuracy of the results could be influenced by the structure of the protein to be analyzed, and its solubility (Marshall and Williams, 1992).

Despite the high Pearson correlation coefficient (0.886) from the range (-1 to +1), only the extraction yield of treatments E4 (pH 11, 0 M NaCl), E7 (pH 12, 0 M NaCl) and E8 (pH 12, 0.25 M NaCl) analyzed by the Kjeldahl method were statistically equal compared with Bradford (Table 2); nonetheless, the other treatments had different behavior depending on the analysis method (P<0.05). It is possible that due to the high pH and the low saline concentrations, the protein was more soluble in those treatments, consequently, the Bradford test was able to quantify the majority of the protein in the sample. However, at lower pH values and higher saline concentrations, the macro-molecule is less soluble and the protein-CBB complex could not be formed.

Table 2. Protein extraction yield.

Treatment	Kjeldahl method	Bradford method
	Yield (g 100 g^{-1})*	
E4	40.41±1.28 Aa	38.11±2.43 Aa
E7	38.79±0.30 Aa	40.28±1.85 Aa
E1	35.01±1.76 Ab	24.01±1.66 Bcd
E8	32.01±1.40 Ac	30.29±0.28 Ab
E9	31.13±1.31 Ac	27.75±1.19 Bbc
E2	27.60±0.09 Ad	18.51±0.12 Be
E5	27.24±0.30 Ad	23.72±1.32 Bde
E3	25.85±0.42 Ad	15.56±0.76 Be
E6	25.56±0.71 Ad	19.09±0.82 Be

*Mean ± standard deviation. Values followed by at least the same capital letter through the rows do not have differences between them by the Tukey test at 5% probability. Values followed by at least the same lowercase letter through the columns do not have differences between them by the Tukey test at 5% probability.

In contrast, the Kjeldahl method determines the total nitrogen content and converts it to the amount of protein by a conversion factor depending on food composition (Mbailao *et al*., 2014), so it is more trustworthy to analyze the protein content in this case. Also, the Bradford methodology has a higher CV than the Kjeldahl one due to the calibration curve of the first one being linear in a small range between 0 ug mL^{-1} to 2000 ug mL^{-1}, so it requires successive dilutions in the sample and that increase the variability (Ernst and Zor, 2010).

As a result of the extraction stage, the treatments E4 (pH 11) and E7 (pH 12) had the best extraction yields (P<0.05). As E4 used less NaOH, it may prevent the damage in the protein from extreme basic mediums (Friedman, 2010). Thus, E4 followed the precipitation stage.

Protein precipitation

There was a significant difference between treatments, and the pH factor had a statistical influence on the precipitation yield (P<0.05) (Table 3). It could be explained because the isoelectric precipitation happens when the pH of the medium is equal to the pI of the protein generating a 0 net charge (Shaw *et al*., 2001).

Table 3. Analysis of Variance (ANOVA) of protein precipitation yield.

Sources of variation	DF	Sum of squares	Mean squares
Total	13	58.53	-
Treatments	4	52.69	13.17*
pH (A)	1	46.83	46.82*
Temperature (B)	1	2.18	2.18 n.s.
Interaction A x B	1	1.46	1.46 n.s.
Curvature	1	2.23	2.23 n.s.
Residue	9	5.83	0.65

* Significant at 5% of probability by the F test. n.s: not significant at 5% of probability by the F test. DF: degrees of freedom.

The CV was 1.21%, which confirms its reliability (<5%) (Condo and Pazmiño, 2015). Table 3 shows that the curvature is not statistically significant (P>0.05), and that implies a lineal model without the need to add extra

experimental points to study the quadratic effect (Gutierrez *et al*., 2008). The treatments P1 (pH 4, 40 °C), P2 (pH 4, 80 °C) and P5 (pH 4.5, 60 °C) were statistically the same and presented the highest precipitation yield *(P*<0.05) (Table 4). The influence of the pH in the reduction of the solubility is related to the achievement of the isoelectric point of the protein. In this condition, the protein-protein interactions increase due to the drastic decline in electrostatic forces and the lesser water interaction with these macromolecules (Santamaría-Fernández *et al*., 2019).

Table 4. Protein precipitation yield.

Treatment	Precipitation yield (g 100 g^{-1})*
P1	68.69±1.88 a
P2	68.50±0.00 a
P5	66.98±0.42 a
P3	64,71±1.07 b
P4	62.81±0.54 b

*Mean ± standard deviation. Means followed by the same letter do not differ between them by the Tukey test at 5% probability.

MO protein had similar behavior to other vegetable proteins, decreasing the solubility when the pH was near 4 (Kobbi *et al*., 2017). The precipitation yield was higher than that reported by Urribarri *et al*. (2004) (62.5 g 100 g^{-1}), who used pH 4 and 50 ºC in *Pennisetum purpureum Schum cv. Mott*.

In general terms, temperature decreases the solubility of the protein due to conformation changes in the structure. The modification is irreversible, and it depends on the temperature and heating time. The solubility of the protein increases until reaching 40 °C and from that point, it starts to decrease. The influence of the temperature is related to the pH and the ionic strength of the medium (Zayas, 2012). This study showed that the pH changed the protein configuration, eliminating the repulsive forces and letting the greater amount of protein in the extract precipitate before it was exposed to the heat treatment, so the effect of temperature was not significant (Tables 3 and 4). Avoiding the use of temperatures while obtaining the best possible precipitation yield prevents the protein from losing heat-labile amino acids or damaging other of its functional properties (Belhadj-Slimen *et al*., 2016).

Considering the average of both stages (extraction and precipitation), the maximum obtained yield was 26.93±0.22 g 100 g^{-1} from the original content of the leaf protein, which represents about 7.26±0.19 g of isolated protein from 100 g of the leaf. This result is similar to those obtained by Nissinen *et al*. (2008) who recovered 24-26 g 100 g^{-1} of *Phleum pratense* leaf protein and to the Edwards *et al*. (1975) results, who reported 26.1 g 100 g^{-1} in *Medicago sativa*. As well as the results from Chiesa and Gnansounou (2011) with alfalfa (*Medicago sativa)* protein (8.53 g 100 g^{-1} from dry leaf). Moreover, Edwards *et al*. (1975) obtained in the same crop a yield of 8.7 g 100 g^{-1} from the dry leaf.

CONCLUSIONS

The best condition for *Moringa oleifera* Lam. leaf protein extraction was pH 11 or 12 without NaCl, which allowed obtaining the highest yield with a significant difference concerning the other treatments. NaCl negatively influenced the solubility of proteins in an alkaline medium. Ranges of pH between 4 and 4.5 are close to the MO leaf protein isoelectric point and have maximized the precipitated protein content with the highest yield. The temperature did not have a significant effect on this process. After both stages (extraction and precipitation), it was possible to obtain up to 26.93±0.23 g 100 g^{-1} of the protein content of the original leaf, which meant 7.26±0.19 g 100 g^{-1} of the dry leaves. This yield was similar to that of the alfalfa concentrates that are generally available on the market. Optimizing the process of extraction and precipitation of protein from MO leaf offers a new option for plant protein that could become a strategy to combat malnutrition in developing countries.

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Antifungal effect from *Zingiber officinale, Aloe vera* and *Trichoderma* sp. for control of *Moniliophthora roreri* in *Theobroma cacao* in Huánuco, Peru

UNIVERSIDAD NACIONAL DE COLOMBIA

Efecto antifúngico de *Zingiber officinale* jengibre, *Áloe vera* y *Trichoderma* sp. para controlar *Moniliophthora roreri* en *Theobroma cacao* en Huánuco, Perú.

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ABSTRACT

Theobroma cacao is the main raw material to produce chocolate, as well as for use in the food, cosmetic, and pharmaceutical industries. However, *Moniliophthora roreri* is one of the most destructive fungal diseases and the main limiting of cacao production worldwide. Thus, this work aimed to assess the inhibitory effect of extracts of *Zingiber officinale* (T1) and *Aloe vera* (T2), and *Trichoderma harzianum* + *Bacillus subtillis*. (T3) on *Moniliophthora roreri* infection in *Theobroma cacao*; in addition, a control (T4) was also evaluated. Each treatment was applied to six plants of cacao. Incidence of monilia infection and fruit weight were monitored every 15 days (in total four periods) after the application of the treatment by spray. Significant differences (P<0.05) were found among treatments for incidence. It was observed that spraying entire cacao trees after two times (approximately 30 days) showed a reduction of monilia infection. After all periods, T1, T2, and T3 showed an incidence of monilia infection by 20.5, 17.7, and 14.9% respectively, compared to cultural control of 41.1%. This reduction of moniliasis infection translates into an increase in fruit weight average for T3 (8.4 kg), T2 (7.3 kg), and T1 (6.9 kg). In contrast, in the control (T3), the fruit weight average decreased by 5.3 kg. Biological control showed efficient management of pathogens as *M. roreri*. It is recommended to use such antifungal (*Aloe vera*) spray over at least 120 days which would decrease infection incidence even more.

Keywords:
Biological control
Moniliophthora roreri
Peru
Theobroma cacao
Fungicides

RESUMEN

El cacao es la principal materia prima para producir chocolate, así como para su uso en las industrias alimentaria, cosmética y farmacéutica. Sin embargo, *Moniliophthora roreri* es una de las enfermedades fúngicas más destructivas y la principal limitante de la producción de cacao en todo el mundo. Así, este trabajo evaluó el efecto inhibidor de extractos obtenidos de *Zingiber officiale* (T1) y *Aloe vera* (T2), y *Trichoderma harzianum* + *Bacillus subtillis* (T3) en la infección producida por *Moniliophthora roreri* en *Theobroma cacao*; además, un cultivo control (T4) también fue evaluado. Cada tratamiento se aplicó a seis plantas de cacao. La incidencia de la infección por monilia y el peso de la fruta se monitorearon cada 15 días (en total cuatro períodos) después del tratamiento por pulverización. Se encontraron diferencias significativas (P<0.05) entre los tratamientos por incidencia. Se observó que la pulverización de árboles enteros de cacao después de dos veces (aproximadamente 30 días) mostró una reducción de la infección por monilia. Después de todos los períodos, T1, T2 y T3 mostraron una reducción en la infección por monilia de 20,5, 17,7 y 14,9% respectivamente, en comparación con el control cultural de 41,1%. Esta reducción de la infección por moniliasis se tradujo en un aumento del peso promedio de los frutos en T3 (8,4 kg), T2 (7,3 kg) y T1 (6,9 kg). En cambio, el control cultural (T3), el peso promedio de la fruta disminuyó a 5,3 kg. El control biológico mostró un manejo eficiente de patógenos como *M. roreri*. Recomendamos el uso de este tipo de antifúngicos (en especial *Aloe vera*) aplicados durante al menos 120 días lo que disminuiría más la incidencia de la infección.

Palabras clave:
Control biológico
Monilia
Perú
Cacao
Fungicidas

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Theobroma cacao (L.) or cacao tree is cultivated mainly in tropical areas of Latin America due to its economic and ecosystem importance (Toala *et al*., 2019). Traditionally, cacao seeds have been exploited for the manufacture of mainly chocolate and candies but based on their organoleptic and nutritional characteristics are used also in food, pharmaceutical, and cosmetic industries (Delgado *et al*., 2018; López *et al*., 2020).

According to the Ministry of Agriculture of Perú (MINAGRI), in Latin America, Brazil, Ecuador, Perú, and Colombia are the countries with the highest cacao production, while Costa de Marfil, Ghana, and Nigeria are the largest producers in the African continent (about 50% of world production) (MINAGRI, 2019). Peru owns 60% of the world´s cacao varieties and its production has been growing at an average annual rate of 15.6% for 10 years consecutively (MINAGRI, 2019). However, in Peru, the moniliasis is the main limiting of cacao production by reducing their production up to 40% (López *et al*., 2020). As consequence, many cacao fields are abandoned or replaced by other more profitable activities (Berget *et al*., 2021).

Cacao production worldwide is limited by fungal diseases, and it is estimated that they cause about 30% of loss and generate an economic imbalance for exporting countries (Delgado-Ospina *et al*., 2021). The most destructive pathogens for cacao are of the genus *Moniliophthora sp*., especially *M. perniciosa* (Dos Santos *et al*., 2020) and *M. roreri* (Bailey *et al*., 2018), which cause moniliasis and the witches' broom disease, both endemic and highly invasive in cacao. Moniliasis caused by *M. roreri* exclusively affects the cacao fruits at any stage of development and may cause losses of up 90% of production (Bailey *et al*., 2018). These fungi reproduce on the cobs and are dispersed by spores that come into contact with other cobs (Tirado-Gallego *et al*., 2016). Symptoms include bumps, premature yellowing or maturation, and oily and necrotic spots, which cause total loss of seeds or a decrease in their organoleptic quality (Joya-Dávila *et al*., 2015).

To control and reduce these diseases, many times farmers have preferred the application of chemicals (Tirado-Gallego *et al*., 2016); nonetheless, its use can generate high costs and cause serious damage to the environment, soil, and human health (Anzules-Toala *et al*., 2021; Torres-de-la-Cruz *et al*., 2019). Other types of control are cultural control (*phytosanitaryness*), agronomic practices, the use of biological agents (especially *Trichoderma* sp*. Bacillus* sp.) of fungus and bacteria since these are friendly to the environment and easy application (Toala *et al*., 2019; Villamil *et al*., 2016; Villamizar-Gallardo *et al*., 2017). For instance, Seng *et al*. (2014) applied *Trichoderma sp*. to control *M. roreri* in Costa Rica and reported a monilia reduction of 11% in only 35 days.

In addition, Joya-Dávila *et al*. (2015) used an extract of *Zingiber officinale* and reported from 88 to 100% control over this pathogen formation and germination. De Rodríguez *et al*. (2005) evaluated the inhibitory effect of *Aloe vera* pulp and liquid fraction on three phytopathogenic fungi (*Rhyzoctonia solani, Fusarium oxysporum,* and *Colletotrichum coccodes*) isolated from a potato crop. Results showed an inhibitory effect on *F. oxysporum* and a reduction in the rate of colony growth. Similarly, Rosca-Casian *et al*. (2007) evaluated the antifungal activity of *Aloe vera* against four pathogenic species *Alternativa* viz., *A. alternat, A. citri,* and *A. tenuissima*, founding significant inhibition on growth and biomass production.

According to the Ministry of Agriculture of Peru (MINAGRI), cacao cultivation is of the largest economic importance in the province of Leoncio Prado. Nevertheless, its production in this province is affected by moniliasis between 12 to 24% (MINAGRI, 2008). Biological control is considered a promising alternative to cope with agrochemicals and plant diseases because is less costly and gives protection to the crop or fruit throughout the crop period. Likewise, their application does not cause toxicity to the plants, is safer for the environment and for the people who apply them. Thus, this work aimed to assess the inhibitory effect of extracts of *Zingiber officinale*, *Aloe vera,* and *Trichoderma harzianum* + *Bacillus subtillis*, in the incidence of *Moniliophthora roreri* on *Theobroma cacao*.

MATERIALS AND METHODS

Study site

The study was carried out at the 14-year-old commercial cacao plantation (CCN-51) during January – February

from 2021 in the Jose Crespo and Castillo district, Leoncio Prado Province, Huánuco-Peru (08°56′00”S; 76°02′30”O). The plantation was located at 540 masl, showing a tropical climate, with annual precipitation of 3179 mm, an average temperature, and relative humidity of 23.8 °C and 86%, respectively (SENAMHI, 2021).

During the study period (3 months) the maximum temperature was 34.4 °C and the average was 30.4±2.4 °C. Likewise, the minimum temperature was 19 °C and the average of 21.5±0.7 °C, average precipitation was13.4±22.3 mm with the highest values among January-February (97.7 mm day^{-1}) (Figure 1).

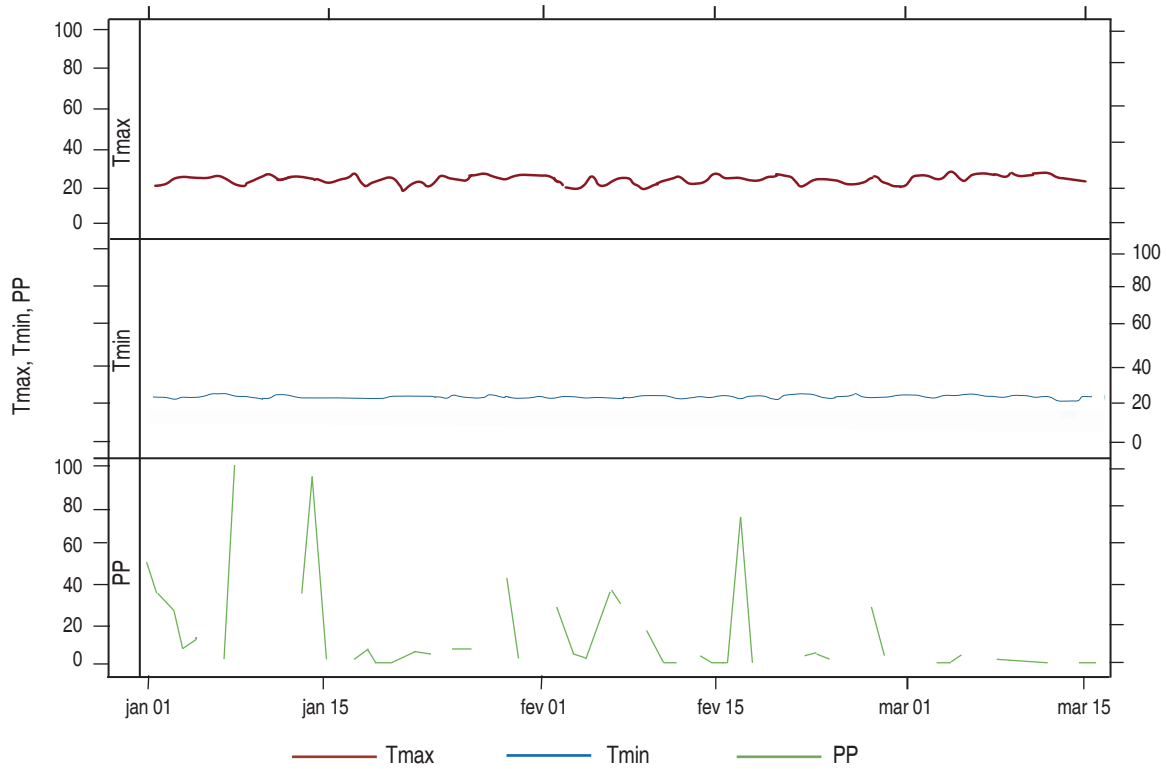

Figure 1. Maximum temperature (Tmax), minimum temperature (Tmin), and precipitation (PP) during the development of the study.

Experimental design

The experiment was conducted employing a random block design (DBCA) with 4 treatments and 3 replications. The experimental area was 630 m^2 with 96 plants of cacao divided into two plots. Each plot had 48 plants (6 columns with 8 plants) at 3x2 m^2. Data were taken from three central plants to avoid the edge effect.

Treatments

In total, four treatments were applied; three biological treatments as fungicides: i) T1: *Zingiber officinale* (it was macerated, and 333 mL of juice obtained was diluted in 20 L of water), ii) T2: *Aloe vera* (it was carried out a mixture of 200 g of *Aloe vera*, 200g of paico leaves (*Dysphania ambrosioides*), 200 g of dried horsetail (*Equisetum arvense*), and 200 g of soap glycerin solid blue, all diluted in 2 L water), iii) T3: a combination (20 L total) of *Trichoderma harzianum* T-22 (w/v, 14 g 10 L^{-1} water) and a biofungicide Serenade® (*Bacillus subtillis* QST173 (v/v, 15 mL 10 L^{-1} water), and iv) T4: cultural control (nothing applied). The cultural control management was applied (sanitary pruning + removal of sick cobs) every 15 days. Treatments were applied throughout four periods in 2021: P1 (11 January), P2 (27 January), P3 (11 February), and P4 (26 February), using a nutrifield PH-B20 manually operated backpack sprayer. Sprays were delivered at a rate of 10 L min^{-1}, wetting the entire trunk, leaves, fruits, and branches from the ground up to 3 m.

Monitoring process

Fruits were monitored for monilia infection on 6 plants;

each plant may contain a number determined of cobs in the four periods: 11 (first evaluation after treatment application) and 27 in the second period in January, and 11 (third period) and 26 (fourth period) in February 2021. At each period, the fruits greater than 15 cm were assessed, considering: i) the number of healthy cobs; ii) the number of cobs affected with *M. roreri*; iii) the number of seeds in healthy cobs, and iv) total weight of the seeds. Before applying any treatment, it was observed that most cobs of cacao were infected by *M. roreri*. Thus, fruits infected totally with monilia were cut and removed, from their trees, burned, and buried to avoid their proliferation (Figure 2). Incidence (%) of cobs harvested with moniliasis were quantified using the following equation: I(%)=(CI/CH)x100. Where I(%): Percentage of fruits infected with the disease; CI: Number of infected cobs; and CH: Number total cobs harvested) (Toala *et al*., 2019). The total weight was calculated by weighing (kg plant^{-1}) the grains extracted from ripe fruits harvested during the period of evaluation.

Figure 2. Cacao fruits: A) infected with monilia, B) Burned, and C) Buried.

Data analysis

Data were submitted to one-way analysis of variance (ANOVA) and subsequent Tukey´s test (value of $P<0.05$ was significant) to compare the mean among different treatments applied. Graphs and statistical analysis were performed using R software, version 3.3.6 (R Team Core, 2019).

RESULTS AND DISCUSSION

This study shows the inhibitory effect of three biological agents (*Zingiber officinale*, *Aloe vera*, and *Trichoderma harzianum* + *Bacillus subtillis*) for the management of moniliasis on cacao fruit in the study area. This work was carried out in the field for 3 months, where biological agents were sprayed (at each start of monitoring) on trees and fruits, and the incidence of moniliasis was monitored every 15 days.

Figure 3 shows the percentage of healthy and infected cobs by *Moniliophthora roreri*, evaluated at each period (P1, P2, P3, and P4) and using three biological treatments: *Zingiber officinale* (T1) *Aloe vera* (T2) and *Trichoderma harzianum* + *Bacillus subtillis* (T3), and control (T4). It was observed among the period an increase of healthy cobs and a reduction of infected cobs. For instance, T1 showed a decrease of infected cobs among periods: 51.9% (P1) to 10% (P2), 20% (P3), and 0% (P4). Likewise, in the P1 (after 15 days of evaluation and the first application of treatments) the T1, T2, T3, T4 were reported that 51.9, 38.9, 45.5, and 55.6% were still infected, respectively. In contrast, in P2 (after 30 days of evaluation and second application of treatments) for T1, T2, T3, and T4 were found that 10.0, 16.6, 0.0, and 44.4% were still infected, but showing an infectious reduction. For P3 (after 45 days of evaluation and third application of treatments) T1, T2, T3, and T4 showed cobs infected of 20, 33.3, 25, and 44.4%, respectively. Finally, P4 (after 45 days of evaluation and third application of treatments) presented cobs infected of 0.0, 6.2, 0.0, and 20% for T1, T2, T3, and T4, respectively. T1 and T3 after four periods of analysis number of infected cobs was eliminated. Nonetheless, T2 showed a minimal presence (6.2%) of *M. roreri,* and T4 (cultural control) kept almost the same number of cobs infected for all periods.

These results showed that the use of biological treatments for moniliasis control can reduce (T2) and eliminate

(T1 and T3) the incidence of this fungal disease present in fruit cacao. Joya-Dávila *et al*. (2015) produced hydro-distilled *Z. officinale* to inhibit the moniliasis infection and reported from 88 to 100% control over their formation and incidence. Besides, Tamayo *et al*. (2016) also reported the effectiveness of *Z. officinale* on moniliasis with a reduction ranging from 40 to 50%. The antifungal or antimicrobial effect of *Z. officinale* corresponds to the gingerol, zingerone, and paradol (bioactive compounds) who contains high flavonoid, phytochemical, and pharmacological contents (Nortaa and Kankam, 2020). These results found in other studies are according to those carried out in this research.

Aloe vera (T2, Figure 3) as fungicide showed a decrease in moniliasis on *Theobroma cacao*. In the scientific literature, there is no reported information on the application of *Aloe vera* and its antifungal and antibacterial effect on moniliasis. However, this bio-fungicide showed successful resulted to control the inhibition of *Mycosphaerella fijiensis* (Jaramillo *et al.,* 2017). Mendy *et al*. (2019) evaluated two types of *Aloe vera* extract against mycelium growth of four pathogenic fungi of papaya fruit: *Fusarium* sp., *Aspergillus niger*, *Colletotrichum gloeosporioides*, and *Lasiodiplodia theobromae*, and was observed a reduction of incidence on papaya fruit after 72 h inoculation. Sitara *et al*. (2011) reported a complete inhibition of *Drechslera hawaiensis* and *Atternaria alternata* and partial inhibition of *Penicilim digitatum* when was applied *Alove vera* gel (0.35%). Likewise, Castillo *et al.* (2010) found efficacy in inhibiting mycelium growth of two common fungi: *Penicillium digitatum* and *Botrytis cinerea* when added *Aloe vera* gel at several concentrations.

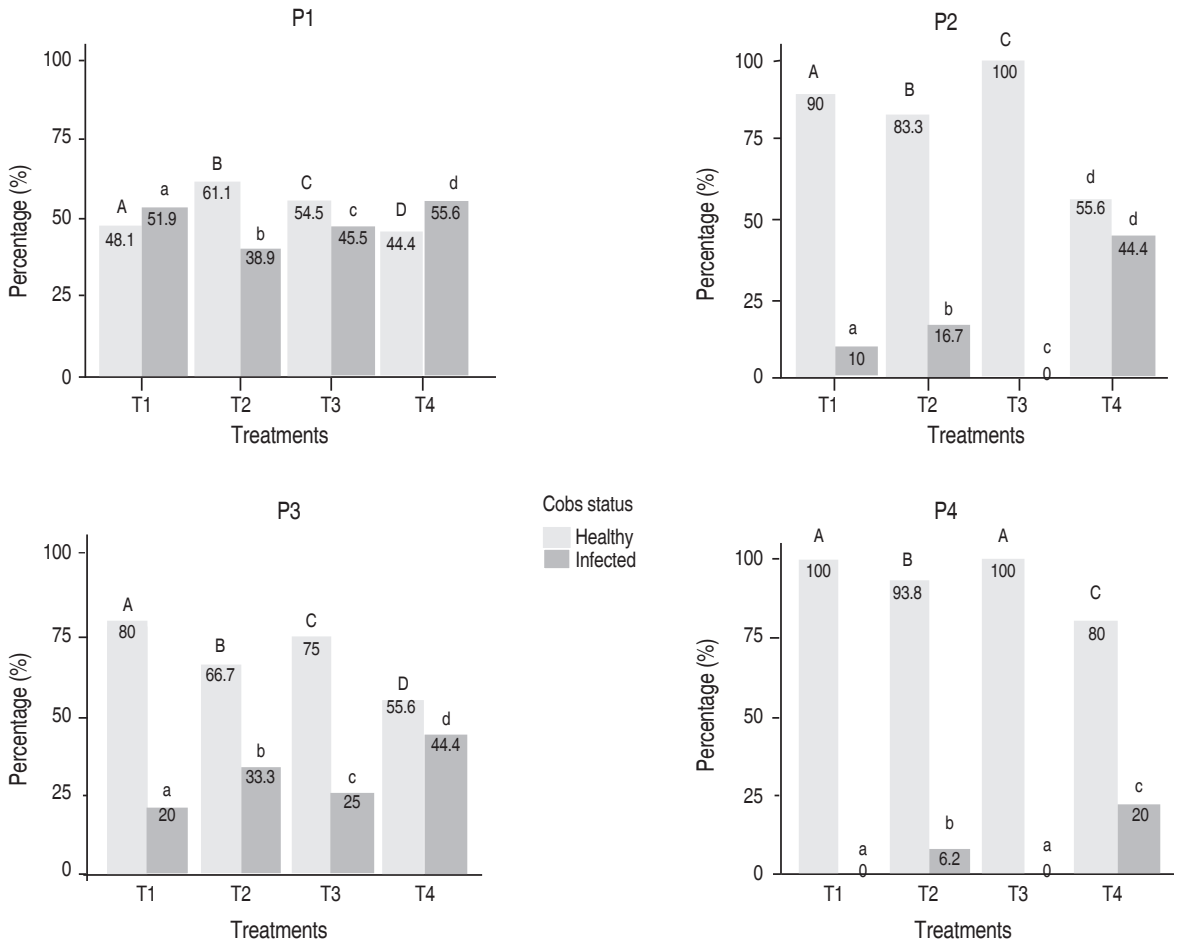

Figure 3. Percentage of healthy and infected cobs by *Moniliophthora roreri*, at each period (P1, P2, P3 and P4) and treatment (T1, T2, T3 and T4).

On the other hand, Seng *et al.* (2014) reported monilia infection reduction by 11% in only 35 days when *Trichoderma sp.* (T3) was used as a spray on the entire cacao tree. This result was similar to the present findings (after 30 days). Likewise, Carvajal *et al.* (2015) assessed the antagonistic activity of two isolated species of *Trichoderma sp.* over *M. roreri* under field conditions and found damage reduction of 19.5 and 11.2% in only 28 days, respectively. However, significant differences among treatments were not reported.

Figure 4 shows the healthy, infected, and total of cobs studied by treatment during all periods. It is noted that the healthy cobs followed this order: T3 (*Trichoderma sp.,* 87.3%) > T2 (80.4%1) > T1 (70.2%) > T4 (65.4%). Significant differences (P<0.05) were found among treatments. The better effect of *Trichoderma sp.,* may be explained because this fungus is a natural antagonist of *M. roreri* and their species produce over 40 different metabolites capable of inhibiting several phytopathogenic microorganisms (Leiva *et al.*, 2020).

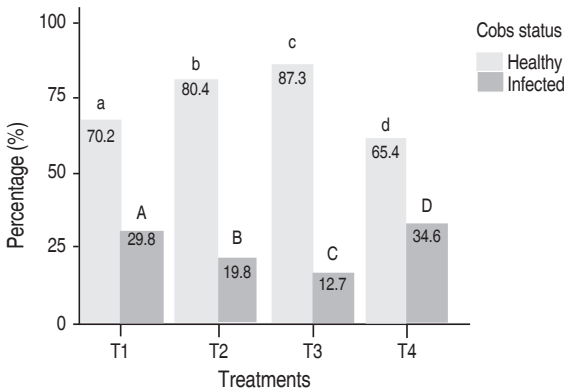

Figure 4. The percentage of healthy and infected by *M. roreri*, monitored for each treatment.

Table 1. Moniliasis incidence (%) and fruit weight (kg plant^{-1}) per period and treatment to control the disease of *M. roreri*.

Treatment	Period	Incidence per treatment (%)	Fruit weight (kg) per treatment	Incidence average (%)	Total fruit weight (kg)
T1	P1	51.9	2.4	20.6 a	6.9 a
	P2	20.5	1.2		
	P3	10.0	1.3		
	P4	0.0	2.0		
T2	P1	38.9	2.3	17.7 b	7.3 a
	P2	16.7	1.0		
	P3	9.1	1.6		
	P4	6.3	2.4		
T3	P1	45.5	0.9	14.9 c	8.4 b
	P2	14.3	0.6		
	P3	0.0	2.1		
	P4	0.0	4.8		
T4	P1	55.6	0.6	41.1 d	5.4 c
	P2	44.4	0.8		
	P3	44.4	1.1		
	P4	20.0	2.9		
Total				24.2	27.9

Values on each column followed by the same letter do not differ significantly (P<0.05).

The infected cobs are ordered of the following manner: T4 (34.6%) > T1 (29.8%) > T2 (19.6%) > T3 (12.7%). Significant differences (P<0.05) were found among all treatments. The control sample was less effective compared to other treatments. Toala *et al*. (2019) reported no control (even was observed an increase of 2% when compared to the initial and final incidence) of this disease when only a control treatment was applied.

Table 1 presents the moniliasis incidence (%) and fruit weight (kg) for each period and treatment and their average on control of *M. roreri.* Incidence of moniliasis average is ordered as follows: T4 (41.1%) > T1 (20.5%) > T2 (17.7%) > T3 (14.9%). Likewise, fruit weight average was: T3 (8.4 kg) > T2 (7.3 kg) > T1 (6.9 kg) > T4 (5.4%). T3 (*Trichoderma* sp. + *Bacillus subtillis*) showed better performance reducing the incidence of moniliasis and obtaining the higher fruit weight. De Sousa *et al*. (2021) evaluated five isolated *Trichoderma* spp., on seed treatment and seedling production of *Theobroma cacao* and found fungi incidence of 26.5% only in the control treatment, while incidence fungi was 0% for all *Trichoderma* isolates. Likewise, Seng *et al*. (2014) reported a significant reduction of incidence of monilia infection by 11%. As well, significant differences (P<0.05) were reported among incidence and fruit weight (except T1 and T2). Increasing fruit weight is probably related to better bio-fungicide control. A similar finding was reported by Trocoli *et al*. (2017) who reported an increase in fruit weight among 30 to 56.5% of pineapple after applying *Trichoderma* sp. on *Fusarium guttiforme*. Likewise, Siswanto *et al*. (2020) revealed better quality and weight of cacao beans after fruit be sprayed by biological and botanical pesticides.

CONCLUSIONS

The results indicated that the use of biological fungicides decreases the incidence of moniliasis and increased fruit weight. T3 (*T. harzianum + bacillus*) showed better performance in decreasing moniliasis infection than T1 (*Zingiber officinale*) and T2 (*Aloe vera*). Likewise, T3 showed a higher increase in fruit weight compared to other treatments. However, *Aloe vera* plant should be studied to apply more different extraction methods with different concentrations to find better antifungal effects.

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Short article

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Selecting optimal parameters for obtaining the extract of red grape pomace

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Selección de parámetros óptimos para la obtención de extracto de uva roja

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ABSTRACT

Due to the industrial processing of grapes, large amounts of by-products are produced. The main varieties of by-products are pomace, which is comprised of skins, seeds and any other solid remaining after pressing process and sediments. It is necessary to implement new effective ways of processing to minimize these residues. This problem is relevant for all the wine-producing countries, including Georgia. It is well-known fact that pomace is an important source of phenolic compounds, which are characterized by high antioxidant activity and possess healing-prophylactic properties. It is also worth mentioning that pomace is an easily spoiled product, and without the proper processing, it cannot be stored for a long time. Thus, this research aimed to obtain optimal parameters for extraction, preserving the antioxidant characteristics. The optimal range of the following parameters for extraction was determined: the temperature for drying 45-50 °C, grinding level 1.5 mm, diluent concentration 70% ethanol/water solvent, extraction module 1:20, extraction temperature 50-55 °C, and duration 2 h. This determination of technological parameters of extraction was done according to the best physical-chemical measures and antioxidant activity level. The physical-chemical tests were performed according to the European Union standards. These parameters can produce an extract with distinct antioxidant characteristics that can be used in the food industry as a natural antioxidant. The extract with distinct antioxidant properties was obtained, which can be used in the food industry as a natural antioxidant.

Keywords:
Anthocyanins
Antioxidant activity
Extraction parameters
Flavonoids

RESUMEN

Debido al procesamiento industrial de la uva, se producen grandes cantidades de subproductos. Las principales variedades de subproductos son el orujo, que se compone de cáscara, semillas y cualquier otro sólido que quede después del proceso de prensado y sedimentos. Es necesario implementar nuevas formas efectivas de procesamiento para minimizar estos residuos. Este problema es relevante para todos los países productores de vino, incluido Georgia. Es bien sabido que el orujo es una fuente importante de compuestos fenólicos, que se caracterizan por una alta actividad antioxidante y poseen propiedades curativas-profilácticas. También vale la pena mencionar que el orujo es un producto que se estropea fácilmente y, sin el procesamiento adecuado, no se puede almacenar durante mucho tiempo. Por lo tanto, esta investigación tuvo como objetivo obtener los parámetros óptimos de extracción, conservando las características antioxidantes. Se determinó el rango óptimo de los siguientes parámetros para la extracción: temperatura de secado 45-50 °C, nivel de molienda 1,5 mm, concentración de diluyente 70% etanol/agua solvente, módulo de extracción 1:20, temperatura de extracción 50-55 °C y duración 2 h. Esta determinación de los parámetros tecnológicos de extracción se realizó de acuerdo con las mejores medidas físico-químicas y nivel de actividad antioxidante. Las pruebas físico-químicas se realizaron de acuerdo con los estándares de la Unión Europea. Se obtuvo un extracto con altas propiedades antioxidantes, que puede ser utilizado en la industria alimentaria como antioxidante natural.

Palabras clave:
Antocianinas
Actividad antioxidante
Parámetros de extracción
Flavonoides

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By-products of winemaking might remain underutilized, causing the loss of large amounts of wholesome substances. Extracts from these residues can be used in food technology to increase the antioxidant levels of the product (García-Lomillo and González-San José, 2016). The chemical composition and antioxidant characteristics of those extracts depend on correctly choosing the extraction parameters (Pazir *et al*., 2020). In order to increase the shelf-life of products, antioxidants are used, which are usually synthetic due to their low price (Li *et al*., 2014). The impact of these kinds of antioxidants is not fully studied, but according to some research, synthetic antioxidants can not only harm the body but also provoke the development of serious illnesses e.g., allergic reactions induced by synthetic antioxidants in children are frequently. The risk group consists of people who previously had allergies to other substances. It can also trigger the development of oncological disease (Atta *et al*., 2017). Natural ingredients demand is continually increasing in the market, and the substitution of synthetic ingredients for natural analogs is a priority in the modern food industry (Mitterer-Daltoe *et al*., 2020).

The development of the food and the processing industry depends to a great extent on the rational use of natural resources of plant origin and the creation of new products (Knorr *et al*., 2020). When processing natural raw materials, maintaining the beneficial properties of the product is a crucial factor. This is closely linked to the optimization of technological processes and regimen of food processing (Jelley *et al*., 2016).

Interest in the by-products of wine production has increased tremendously. There are numerous studies about the usage of red grape processing by-products in the food industry, particularly pomace (Charalampia and Koutekidakis, 2016). This product has captured scientists' attention because of its high levels of biologically active compounds (Teixeira *et al*., 2014). Traditionally, pomace was used to produce food colorants and grape seed oil. Recent studies are directed to increase the beneficial characteristics and nutritional values by adding extracts of biologically active compounds in the foods (Iuga and Mironeasa, 2020). Pomace is usually utilized as an antioxidant, food coloring, and antimicrobial agent since it is rich in phenolic compounds (Luchian *et al*., 2019).

There are many extraction methods to produce an extract rich in new biologically active compounds from by-products; however, to maintain valuable components, an individual approach is needed since the preservation of the chemical content and the antioxidant characteristics depends on whether the extraction was carried out correctly or not. (Ju and Hovard, 2003).

In this context, this study aimed to determine optimal extraction parameters to produce an extract, which would be rich in antioxidants from pomace. In addition, this study shows an alternative to recycle wine waste and replace synthetic additives in food with natural ones, reducing environmental risk.

MATERIALS AND METHODS

Rare Georgian red grape varieties were used (Simonaseuli, Adreuli Shavi Sreluri, Gabasha, and also worldwide known Saferavi). The samples were taken from the Base of Permanent Crop Research Located in Jigaura, Georgia.

These samples are the secondary product of wine production. The temperature of the grape juice was 18 °C, alcoholic fermentation was carried out at 21-22 °C, in moisture content of 75-85%, the grape juice was stirred before and during the fermentation 4-5 times a day. Fermentation lasted 12 days and a maceration process of 5 days.

The pomace was removed after the completion of the alcoholic fermentation of the wine and pressed under a hydraulic press, resulting in moisture content of 37%. The grape pomace was stored at -20 °C. Before the experiment, it was defrosted at room temperature. The pomace samples used in the experiment are products obtained after alcoholic boiling. The determination of technological parameters of extraction was conducted according to the results obtained from the analysis conducted on the mixture of equal amounts of all the pomace samples (Luchian *et al.,* 2019).

Scientific research was conducted at Georgian Technical University, in the research laboratory of the Food Technology Department of Agrarian Sciences and Biosystems Engineering Faculty. Physical-chemical research was performed following the European Union Standards. The results given in the tables were obtained by calculating the average of the results attained from

three test repeats. For the statistical processing of the test results was used MS Excel 2019.

One of the methods of preserving pomace is the drying process (Goula *et al.*, 2016). In order to determine the optimal temperature, the drying process was conducted in the drying cabinet at different ranges of temperature: 45-50 °C, 95-100 °C, and 125-130 °C. After drying, chemical consistency (total phenols, flavonoids, anthocyanins, tannins) and antioxidant activity (ability to catch free radicals of DPPH 2.2'diphenil – 1 – picrylhydrazyl) of the pomace were observed (Kedare and Singh, 2011).

To determine the grinding level of pomace, the dried sample was ground in the laboratory mill to get the particle sizes of 0.5, 1, 1.5, 2 and 5 mm. Extraction was done via the retention method at 22 °C. The experiment was performed by using dried and ground raw material, which was placed in Erlenmeyer flasks, 20 g in each; 50% ethanol was added for the extraction and was stored for 24 h. After this process, the amount and antioxidant activity of extracted phenolic compounds were measured (Amendola *et al.*, 2010).

To determine the extraction module, 50% ethanol with the ratios of 1:5; 1:10 and 1:20 were poured onto the pomace (raw material), which was ground into 1.5 mm particles and dried at 45-50 °C, and then, it was kept at 22 °C for 24 h. After this process, the amount and antioxidant activity of extracted phenolic compounds were measured.

One of the important factors in the extraction process is the nature of the solvent. The use of permissible extracts in the food industry creates the necessary conditions for the use of a solvent that is harmless to human health. There is a relationship between the nature of the extraction solvent and the antioxidant properties of grape pomace extracts (Yilmaz and Toledo, 2006). In order to identify the optimal diluent, a solvent with the ratio of 1:10 was poured onto the pomace (raw material), which was ground into 1.5 mm particles and dried at 45-50 °C. Usually, water, ethanol, and ethanol-water solutions at varying ratios were used as a solvent (water, ethanol, and 30%, 50%, 70% ethanol-water solutions). The mixture was kept at 22 °C for 24 h while being periodically stirred. After the extract was separated from raw material, the quantity and antioxidant activity of total phenols, flavonoids, anthocyanins and tannins were measured.

To determine the optimal extraction temperature, three variations of temperature were observed: 35-40 °C, 50-55 °C, 75-80 °C. As a diluent, 70% ethanol: water solution was used. The total amount of phenols, flavonoids, anthocyanins and tannins was measured in equal circumstances after retaining the mixture for 1 h.

To determine the extraction time, 70% ethanol was used. Extraction was conducted at the optimal temperature of 50-55 °C for 1, 2, 3, and 4 h. As in the previous procedures, the amount of extract obtained and antioxidant activity were measured.

RESULTS AND DISCUSSION

Khanal *et al.,* 2010 studied the effect of heating on the stability of grape and blueberry pomace phenolic compounds. Their study showed that heating at temperatures higher than 125 °C, the phenolic compounds suffer a considerable loss.

Lin and Chou (2008) studied effect of the heat treatment on total phenolic and anthocyanin contents of fermented black soybeans. The results of the study showed that 40-100 °C heating reduced the total phenolic and anthocyanin content. According to the results of the present study, it is posible to conclude that an increase in temperature has a negative effect on the content of phenolic compounds.

The chemical composition of the product was determined after drying (Table 1). Thermal processing does affect the chemical composition and oxidative activity of pomace. It also significantly increases the concentration of phenols, flavonoids, anthocyanins and tannins (Carmona-Jiménez *et al.,* 2018). This can be due to several factors: 1. The plant cells break down under the influence of high temperature, which makes easier the extraction of phenolic compounds and flavonoids. 2. As usual, phenolic compounds in plant cells are connected to sugars in form of glycosides. After affecting the cell with a high temperature, the glycoside connections are split, and the phenols are released from the cell. The phenols and flavonoids freed in the above-explained process maintain high antioxidant activity.

By thermal processing, pomace antioxidant activity was decreased compared to the raw pomace, which can be explained by the factor that, in addition to phenols and

Table 1. Phenolic compounds regarding the temperature of drying process of pomace.

Drying °C	Total Phenols g 100 g^{-1} dry material	Total Flavonoids g 100 g^{-1} dry material	Total Anthocyanins mg 100 g^{-1} productivity	Tannins mg 100 g^{-1} productivity	Antioxidant activity %
Raw Pomace	2.79±0.02	2.32±0.04	737.0±0.9	10.51±0.8	79±0.9
45-50 °C	3.31±0.18	3.01±0.03	682.0±1.3	82.40±1.3	64±1.1
95-100 °C	2.85±0.03	2.98±0.06	101.7±1.2	72.20±1.5	61±1.3
125-130 °C	2.29±0.02	2.11±0.04	223.2±0.8	49.20±0.8	51±1.3

flavonoids, plant cells contain vitamins C, A, E, as well as the fermentation system of the cell, which also represents an antioxidant. When affected by heat, these substances have degenerated, and only the antioxidant activity of phenolic compounds is held intact. Kurozawa *et al.,* 2014 studied the degradation of vitamin C in papaya fruits at different drying temperatures and found that increasing the temperature significantly reduced the amount of vitamin C.

At the temperature of 45-50 °C dried pomace contains a larger amount of phenols, flavonoids, anthocyanins and tannins. Apart from that, the mentioned temperature also results in the best antioxidant activity. Based on the results obtained in this experiment, 45-50 °C was chosen as the optimal temperature range for drying the grape processing by-products for 24 h. Below 45 °C and above 130 °C, the extraction of phenolic compounds was minimal, regarding the duration, a decrease in phenolic compounds was also observed when drying for more than 24 h.

The results of the physical-chemical analysis were performed to determine the optimal grinding level of pomace (Table 2).

Table 2. Phenolic compounds after grinding process of dried pomace.

Grinding Level mm	Total Phenols g 100 g^{-1} dry material	Total Flavonoids g 100 g^{-1} dry material	Total Anthocyanins mg 100 g^{-1} productivity	Tannins mg 100 g^{-1} productivity	Antioxidant activity %
0.5	2.01±0.01	2.11±0.02	625.1±1.2	9.61±1.4	31±0.9
1	3.15±0.03	2.84±0.04	668.7±1.4	71.98±1.2	60±1.1
1.5	3.62±0.04	3.19±0.06	912.9±0.7	68.41±1.3	64±1.2
2	2.19±0.03	2.03±0.03	216.8±0.9	48.0±0.9	30±0.9

The maximum productivity of extraction was reached when the grinding level was 1.5 mm, due to the maximum surface contact between solid/solvent. An increase in the grinding levels caused a decrease in the solvent adsorption capacity and, consequently, a decrease in the extraction quality according to the number of phenolic compounds and antioxidant activity. Brewer *et al*., 2013 studied the wheat bran particle size influence on phytochemical extractability and antioxidant properties and showed that the coarse treatment exhibited significantly higher antioxidant properties than the fine treatment.

Physical-chemical analysis of the produced extract was performed to determine the extraction module using three variations of pomace/diluent ratios (Table 3). Physical-chemical analysis of the produced extract was performed to determine the optimal diluent concentration using diluents with three different concentrations (Table 4).

Dimcheva *et al.,* 2018 studied the effect of the solid/solvent ratio on the total flavonoid, polyphenol, anthocyanin contents, and TEAC of ethanolic extracts from the grape seeds and pomace. They found that the maximum yield of phenolic

Table 3. Impact of pomace extraction module on phenolic compounds.

Extraction Module	Total Phenols g 100 g^{-1} dry material	Total Flavonoids g 100 g^{-1} dry material	Total Anthocyanins mg 100 g^{-1} productivity	Tannins mg 100 g^{-1} productivity	Antioxidant activity %
1:5	2.12±0.17	2.23±0.04	658.8±0.8	35.27±1.2	41±1.2
1:10	3.62±0.25	3.19±0.03	912.9±0.9	68.41±1.1	64±1.1
1:20	3.63±0.03	3.20±0.02	913.1±1.3	68.42±1.3	64.2±1.3

Table 4. Impact of diluent concentration on the pomace extraction productivity.

Diluent	Total Phenols g 100 g^{-1} dry material	Total Flavonoids g 100 g^{-1} dry material	Total Anthocyanins mg 100 g^{-1} productivity	Tannins mg 100 g^{-1} productivity	Antioxidant Activity %
H_2O	2.11±0.01	1.30±0.04	339.9±1.4	14.36±1.3	32±0.9
30% C_2H_5OH	3.24±0.04	3.12±0.06	671.8±1.1	42.89±1.4	47±1.1
50% C_2H_5OH	3.62±0.02	3.19±0.02	912.9±1.5	68.41±1.4	51±0.8
70% C_2H_5OH	4.98±0.02	3.35±0.03	1167.1±0.7	72.90±1.6	68±1.3
C_2H_5OH	4.15±0.01	3.05±0.05	610.1±0.9	69.49±1.1	56±1.2

compounds was reached at 1:20 of the grape pomace and solvent ratio. Similarly, the same ratio of 1:20 was found to be optimal for the present study. There was a difference when using the solvent concentration. Dimcheva *et al.* (2018) used a 50% ethanol /water mixture as a solvent. The present research showed that the best extraction rate was 70% ethanol/water.

As a result of the experiment, the best performance of phenols, flavonoids, anthocyanins and tannins extraction from pomace was when 70% ethanol: water solution was used as a diluent.

Extraction temperature has an important influence on the extraction process. It is known that according to the temperature, the state of procyanidin complexes in the pomace can be changed.

To determine the optimal extraction temperature, three temperature ranges were investigated: 35-40 °C, 50-55 °C, 75-80 °C. Solution of 70% alcohol/water was used as the solvent. Total phenols, flavonoids, tannins and anthocyanins were determined under the same conditions after 1 h.

Table 5. Correlation between chemical composition and extraction temperature.

Extraction temperature °C	Total Phenols g 100 g^{-1}	Flavonoids g 100 g^{-1}	Anthocyanins mg 100 g^{-1}	Tannins mg 100 g^{-1}	Antioxidant activity %
35-40	3.47±0.05	2.88±0.03	986.9±1.3	72.49±1.4	47±1.2
50-55	3.79±0.02	3.29±0.05	813.3±1.1	75.27±1.3	64±1.1
75-80	3.71±0.03	3.25±0.01	596.9±1.1	69.86±1.2	51±0.9

Based on the results, it can be concluded that the optimal temperature of pomace extraction is 50-55 °C; however, a lower temperature is more convenient for anthocyanins, given that by increasing the temperature, the amount of anthocyanins decreases in response. A further increase in temperature does not increase

the extraction integrity and destroys anthocyanins. Therefore, in this case, increasing the temperature is inexpedient. A similar experiment was carried out by Khanal *et al.* (2010), who found that a increase in the temperature causes a decline in the amount of anthocyanins.

In conclusion, the best pomace extraction temperature is 50-55 °C. Following the increase in temperature does not contribute to producing the best version of the extract; it only decomposes its chemically active substances such as polyphenols and vitamins. Consequently, no further increase in temperature is recommended in this case. Furthermore, 50-55 °C was the best temperature range to maintain the antioxidant activity as well.

Table 6 shows the extraction of total phenols, flavonoids, anthocyanins and tannins; the highest antioxidant activity was achieved during the extractions with a duration of 2 h. The analysis showed that the technologically justified duration of extraction for grape extractions is 2 h, a longer extraction leads to the destruction of phenolic substances. The optimal parameters to produce the extract are summarized in Table 7.

Table 6. Chemical composition of the extract concerning the extraction time.

Extraction duration (h)	Phenolic substances g 100 g^{-1}	Flavonoids g 100 g^{-1}	Anthocyanins mg 100 g^{-1}	Tannins mg 100 g^{-1}	Antioxidant activity %
1	3.79±0.02	3.29±0.05	0.8133± 0.9	75.27± 1.5	64±0.9
2	4.01±0.03	3.51±0.07	0.8251±1.1	79.40±1.4	68±0.7
3	3.91±0.02	3.45±0.06	0.8217±1.4	78.03±1.3	42±0.5
4	3.80±0.04	3.35±0.02	0.8191±1.5	76.26±1.5	38±0.7

To sum up, the extracts produced were distinguished by a high concentration of phenolic compounds, flavonoids and tannic substances and were characterized by high antioxidant activity. The data obtained from the study are not inferior to those conducted by other authors as Raiba *et al.* (2014), who recommend its use in the food industry.

Table 7. Chemical composition of the extract produced.

Dry substance %	Tartaric acid %	Total Phenols %	Flavonoids mg 100 g^{-1}	Anthocyanins mg 100 g^{-1}	Tannins mg 100 g^{-1}	Antioxidant activity %
17.5±0.12	8.0±0.01	4.73±0.01	4.0±0.06	437±0.08	87±1.2	80±1.3

CONCLUSION

The technology regimen of experimental pomace extraction was determined. This regime was selected for raw material: drying temperature 45-50 °C for 24 h, extraction temperature 50-55 °C, extraction duration 2 h. Concentration was done via vacuum. It was identified that by following the mentioned regimen, the extract stood out beacuase of its high concentration of phenolic compounds, flavonoids, and tannic substances. Due to this, it can be used in food technology as an antioxidant. Also, according to the performed tests, it can be concluded that the produced extract can be utilized as a natural food coloring due to its high concentration of anthocyanins. Further studies should be performed to determine in which foods can maintain the given color its stability, also what would be the advisable amount of it that can be used to replace synthetic food dye with the natural analog.

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Agri-food safety optimized by blockchain technology: review

Seguridad agroalimentaria optimizada por medio de la tecnología blockchain: revisión

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ABSTRACT

Blockchain technology is a distributed database, an innovation tool in the agri-food supply chain in processes such as production, distribution, marketing. In this research work, the blockchain technology application in agri-food security processes was evaluated, establishing the best conditions for its adoption in companies, this proposal synthesizes the contributions as a disruptive technology, for this, the information was collected from the period 2018-2020 from the Scopus and Web of Science bases, performing an analysis of the information using the Atlas TI 8.4 software, establishing the focus of the research on a network of codes suggested by some authors. It was found that the contribution of Blockchain for the years of study was traceability 26%, supply chain 17.5%, technological development 10.4%, trust 9.8%, among others. It is concluded that establishing the theoretical link between technology and traceability processes in supply chains, traceability in the agri-food sector is essential to certify information of interest to the stakeholder group. This is because traceability is the transcendental element of the food safety system that allows guaranteeing control in the supply chain when processes are being recorded and enriching the databases, which can be available to the final consumer to check the details of the production cycle and that technological elements generate competitiveness in companies with blockchain in their procedures, promote high levels of transparency, data security, decentralization, among other terms associated with trust, and a better relationship with the consumer is developed and a greater number of sale increasing profitability.

Keywords:
Agri-food sector
Blockchain technology
Food supply
Health surveillance
Traceability
Trust

RESUMEN

La tecnología Blockchain es una base de datos distribuida, una herramienta de innovación en la cadena de suministro agroalimentario en procesos como: producción, distribución, mercadeo. En este trabajo de investigación documental se evaluó la aplicación de la tecnología Blockchain en los procesos de seguridad agroalimentaria, estableciendo las mejores condiciones para su adopción en las empresas, esta propuesta sintetiza los aportes como tecnología disruptiva, para ello se recolectó información entre 2018 -2020 a partir de las bases Scopus y Web of Science, realizando un análisis de la información utilizando el programa Atlas TI 8.4, estableciendo el foco de la investigación en una red de códigos sugeridos por algunos autores. Se encontró que el aporte de Blockchain para los años de estudio fueron trazabilidad 26%, cadena de suministro 17.5%, desarrollo tecnológico 10.4%, confianza 9.8%, entre otros. Se concluye que, estableciendo el vínculo teórico entre tecnología y procesos de trazabilidad en las cadenas de suministro, la trazabilidad en el sector agroalimentario es fundamental para certificar información para el grupo de interés. Esto porque la trazabilidad es el elemento trascendental del sistema de seguridad alimentaria que permite garantizar el control en la cadena de suministro cuando se están registrando los procesos y enriqueciendo las bases de datos, que pueden estar a disposición del consumidor final para verificar los detalles del ciclo de producción y que los elementos tecnológicos generen competitividad en empresas con blockchain en sus trámites, que promuevan altos niveles de transparencia, seguridad de datos, descentralización entre otros términos asociados a la confianza y se desarrolle una mejor relación con el consumidor y mayor número de ventas aumentando la rentabilidad.

Palabras clave:
Sector agroalimentario
Tecnología blockchain
Suministro de alimentos
Vigilancia de la salud
Trazabilidad
Confianza

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INTRODUCTION

The world economy is undergoing dramatic changes, driven largely by the availability of digital information at high speed that allows the exchange of assets quickly, in real-time, and with remote sensors that generate a line of traceability, blockchain technology along with other digital tools are currently making inroads in the financial, pharmaceutical, agri-food, and other sectors. The generation of knowledge of ICT (Information and Communication Technologies) is in its initial stage. This research evaluated the application of blockchain technology (BT) in the agri-food supply chain, the strengths of the implementation of blockchain technology in agri-food innovation processes, and the influence of BT on the competitiveness of agri-food supply chains. This technology is constantly growing, the dynamism of its applications is of interest to businessmen, entrepreneurs and research and innovation personnel because they are in constant search of its implementation in the production, distribution, marketing processes and consumption of raw materials, by-products, and processed foods (Saberi *et al.,* 2019; Torky and Hassanein, 2020).

The information generated by economic transactions, the exchange of information and product tracking, creates a traceability system, which allows the end customer to perform a reliable control of the food or service. Blockchain technology establishes commitments, business, audits, certifications, or validations in real-time, with simultaneous verification of information by users. Advances in this monitoring system would allow those in the supply chain to establish more rigorous control, indicating improvements in quality and safety standards. Systematic adoption in the agricultural sector would help in local, national and international negotiation processes, establishing a reliable and dynamic intervention with instant feedback, to speed up a decision making (Feng *et al*., 2020; Mahyuni *et al*, 2020).

The pace of technological and business innovation has increased over the last few years, making it difficult to develop business models that maintain sustained profitability over time. It should be emphasized that the internet enables the creation of new business models with instant global reach (Alvarez, 2018).

Blockchain technology after cryptocurrencies and their transactions without the need for an intermediary and in a secure manner, passed to the second generation through smart contracts, allowing to establish the relationship through external conditions that are executed or not. Subsequently, the third generation of blockchain arrived, which consists of decentralized applications. These generations use the blockchain with the internet of things (IoT), in such a way that sensors are utilized to transmit information to the blockchain and allow smart contracts to be executed. Also, blockchain technology could work together with artificial intelligence establishing a possible fourth-generation (Beltrán, 2020).

To estimate the relationship between innovation and profitability, a cluster analysis was performed, using the benefit ratio: cost of productive activity studying adoption and speed in innovation processes. The highest rates of innovation are related to the production and conservation of natural resources (75%), allowing farms with ancestral production and greater innovation to present higher profitability (Espejel *et al*., 2019).

The use of productive programs through networks that facilitate greater integration and partnerships between companies is one of the main pillars to sustain competitiveness, and the generation of an enabling environment for business are some indicators of interest in business competitiveness for the Organization for Economic Cooperation and Development (OECD) (Ibarra *et al*., 2017).

By optimizing the production chain of the agricultural sector in Colombia will be possible to take advantage of agro-industrial waste, reducing environmental impacts with the reuse of potentially polluting waste and with high disposal costs, also by gradually reducing the consumption of fuels from petroleum, emission of CO_2 and other pollutants; additionally, the optimization of the production cycle may generate employment at each stage, favoring unskilled labor and rural sectors, boosting the growth of the agricultural areas (Duque and Fúquene, 2020).

BT is an emerging technology with initial development and little experience of incorporation in supply chain companies. These companies developed Origin Chain, which is a blockchain-based traceability software that restructures the current system by replacing the

central database with blockchain. Both qualitative and quantitative analysis of Origin Chain's software was also demonstrated. Based on our experience and analysis, finding that the structural design of smart contracts has a great impact on the quality of the system (Xu *et al*., 2019).

Likewise, Eskardillo computer technology (ESK), is a tool with a smartphone terminal that is based on three principles: i. systematic recording of individual data (milking control, productivity, genetic merit, morphology), ii. phylogeny, processing and interpretation of big data and iii. interactive feedback to the farmer to optimize animal selection. Unquestionably, the management of big data favors the decision-making process to optimize modern enterprises to maintain sustainable intensification (Belanche *et al*., 2019).

Applications such as iSolve or BlockRx and their private companies look to the blockchain to develop proprietary technologies, it is interesting to underline decisions that seek to optimize the daily operability in markets, the protection of confidential information and the development of new drugs or the efficient distribution of drugs along the entire supply chain. It is also important the advantage of drug origin verification, through the implementation of smart contracts leaning towards new services (Preukschat *et al*., 2017).

Vertical integration is a type of supply chain governance with research opportunities for the implementation of sustainable approaches (corporate social responsibility, green supply chain management, industrial ecology, stakeholder theory, circular economy and sustainability science), mainly in small and medium-sized poultry supply chains. Furthermore, Brazilian environmental legislation still needs to be revised to align with the Sustainable Development Goals (SDGs) because the legislation will not guarantee sustainable management and efficient use of natural resources (Pohlmann *et al*., 2019). It is essential for poultry farmers to systematically collect data from the production environment to be successful in their production activities, where a sensor network was used to record poultry management data, which is then preprocessed using machine learning techniques. The results obtained were validated and compared with the action plans generated by a human specialist and bibliographic references. The analysis suggested that the action plans derived from the proposed model follow with acceptable accuracy (Ribeiro *et al*., 2018).

Studies show that to obtain greater competitiveness, companies should make improvements such as the search for new markets, project more work on achieving bar codes for all products, staff training programs, take advantage of support programs both for the acquisition of equipment and its management and use, according to current requirements and trends, and seek alternatives for collective certifications such as designations of origin, which can be more economical, but with recognition and value-added (Torres *et al*., 2020).

The growth of the industry in different sectors makes Colombia an economic power of reference in the region. For this reason, the different productive sectors must generate initiatives aimed at innovation and implementation of new technologies such as blockchain to make a competitive leap in global markets (Cardona and Orozco, 2019).

Likewise, the analysis conducted allowed testing that investments in innovation have caused an increase in the productivity of small and medium-sized companies in Mexico. Investment in research and development (R&D) did contribute positively to investment in innovation. The small and medium-sized companies that invest the most in innovation are not only those with the largest budgets or the most R&D plans but also those that regularly carry out technological activities, such as acquiring licenses, updating processes, modifying products or developing their technologies. Innovation is a competitive advantage for companies with the skills to capitalize on it. Lately, it has been recognized that there are relentless and triumphant innovation processes in small and medium-sized enterprises in Latin America (Kato-Vidal, 2019).

Innovation, known as digital opportunity trust (digital opportunity trust, DOT) is an entrepreneurship solution to address difficulties in the agricultural market, innovation based on the synthesis of tacit knowledge (knowledge based on experience in agriculture and agribusiness) and codified knowledge (based in computer programming) builds the space of innovation opportunities to positively address agricultural problems (Relf-Eckstein *et al*., 2019).

The supply chain for agribusiness products is in an incipient stage, with isolated operational problems, with little attention to their interdependence, which can be attributed to the fragmented nature of the supply chains themselves and the lack of integrated transportation and information infrastructure. The main factors related to successful integration are human resources, organizational strategies, information and information technologies implemented by the organization, with the last two being of more weight according to the authors (Bustillos and Carballo, 2018).

Logistics processes occur at all levels, but especially important are the logistics of procurement and distribution of finished products. An inefficient logistics process can generate a loss of customers and a decrease in both regional and national market share, which ultimately means losses for the company. Larger companies have logistics schemes that guarantee the timeliness and quality of the processes (Benavides-Sánchez *et al*., 2018).

The above background allows evidencing that the emerging blockchain technology is a tool of great interest to innovate in productive and operational processes in the agricultural sector, this multiconnected network with the simultaneous online participation of several users, allows performing a systemic control of each process, reliable transparent management in businesses or companies that transfer and track their commercial procedure.

MATERIALS AND METHODS

The present work was carried out through the qualitative method to understand the concepts of blockchain technology. The information was processed using the specialized analytical recording technique (RAE), and the Atlas TI 8.4 software was used for the analysis. In addition, technical sheets were made to optimize the analysis.

RESULTS AND DISCUSSION

The characteristics of BT need to be evaluated in order to make decisions for the benefit of users. Therefore, the following factors were analyzed in the review:

Traceability. Blockchain technology brings a new hope in quality control systems that ensures traceability from post-harvest and food distribution through nodes with its stakeholders in the value chain (Varghese *et al*., 2019). In addition, it enables traceability of the food and ingredient supply chain is very difficult for retailers in the event of a foodborne illness outbreak.

It can be added that upstream (producers) and downstream (distribution and marketing) members of the supply chain require the development of information bases related to traceability to provide evidence of state regulatory compliance to the most demanding customers and control bodies (Casino *et al*., 2020). Small producers or retailers can monitor the current capacity of distributors and be directly connected to place new orders using the entire traceability system to collect relevant information such as delivery time, batch size, storage and transportation conditions (Kayikci *et al*., 2020).

Trust. Blockchain technology allows the company to generate a relationship of trust with its consumers, through decentralized verification, strategically solving the customer churn that marketing managers face. Also, decreasing costs related to attracting new customers through advertising and promotions (Ramírez, 2020). Technological trust refers to the performance and availability of blockchain by using encryption as the main strength, developing privacy features and security, establishing significant factors for the adoption of blockchain technologies (Gökalp *et al*., 2020). In addition, technology plays a key role in improving collaboration through rapid trust-building among the numerous actors involved in relief operations in case of natural disasters or health issues (Dubey *et al.,* 2020).

Data security. Currently, supply chains have been enhanced to their maximum expression by incorporating technological tools such as social networks where the use of digital platforms for education at all levels, teleworking, procurement of food, pharmaceutical, cleaning products, among others. Especially blockchain technology for the realization of transactions in real time between different designated parties, establishes security and immutable conditions. In addition, blockchain can be used to monitor public health surveillance data (Porcelli, 2020). Food safety can be improved through traceability with blockchain solutions since these can be used to take advantage of the information from each node in the supply chain, this data is more reliable and tamper-proof thanks to the blockchain feature, which guarantees the quality of food (Varghese *et al*., 2019).

Thanks to greater transparency and higher quality of transaction details, blockchain brings improvements in food safety and quality, such as product sustainability and consumer awareness (Tripoli and Schmidhuber, 2018).

Transparency. Blockchain technology is a tool that will represent a new method of trust and transparency without the need for third parties, in addition to generating control, process agility and observation of the data embedded in the nodes. Demonstrating information and trust can be relevant for the growth of companies, since it allows to substantially change the operation of the world economy and industry today (Avila, 2018; Vargas, 2018). Blockchain technology can evidence some benefits for consumers such as product traceability and information transparency. This is particularly useful, especially in agri-food companies, where customers are concerned about the composition and its effect on the organism, demonstrating the origin of products and components, with ethical and responsible production (Cardona and Orozco, 2019). In addition, it can be established that food safety, value chain efficiency and transparency are effectively improved by ensuring high transparency, stability and reliability of the agri-food value chain. Finally, it can provide liquidity, more accurate record-keeping and high transparency of ownership (Zhao *et al.*, 2019).

Decentralization. Blockchain is the newest technology, it is a distributed digital that can record transactions in multiple sets of blocks. It is a decentralized system that does not rely on a single entity for safekeeping, which ensures security (Varghese *et al.*, 2019). Key technological components such as communication protocol decentralized storage, smart contracts and cloud are necessary to build a blockchain system (Zhao *et al.*, 2019). Also, blockchain-based transactions are being tested in many sectors, such as finance, manufacturing, energy, and government. They are also being used in relation to agri-food supply chains. The adoption of this technology promises to offer a transparent, decentralized and secure transaction process and can reduce transaction costs (FAO, 2019). In addition, BT with its technological infrastructure allows databases to be decentralized and updatable in real-time, thus allowing applications, proprietary and third-party services to be within reach of mobile devices, solving new customer needs (Pino and Prado, 2019).

Factors influencing the implementation and competitiveness of the supply chain

The results of the documentary research on the application of blockchain in agri-food safety processes show that several factors allow strengthening the implementation and influence the competitiveness of the supply chain.

In Table 1, it is recorded that most of the authors established that traceability (26%) was the main term to determine conditions for initial adoption of the blockchain system in their companies, considering that the digital traceability of products is a modern tool to know the conditions of each input, product or service. The second factor reported with the greatest influence is the supply chain (17.5%) where processes are enriched with various details gathered in databases, which can be available to customers or consumers to verify the specific information of each product. In third place, technological development (10.4%) is observed as an influencing factor with the support of information and communication technologies: big data, cloud, internet of things, radio frequency identification, data analysis among others, since applications integrated into the blockchain can allow companies to manage production processes and promote new levels of competitiveness and profitability in the agri-food sector.

The term trust (9.8%) ranks as the fourth most important factor according to the authors, because trust is associated with transparency, data security, decentralization and interoperability, which together add up to (29.1%) that allow real-time monitoring of stakeholders. This partnership develops a high level of security among producers, traders and consumers to develop and conclude various negotiations within the supply chain.

Strengths of blockchain implementation in agri-food innovation processes.

In the following section, the strengths of the implementation of blockchain technology in innovation processes were evaluated (Table 2), identifying the importance of each code establishing an interaction between the following terms: trust, data security, transparency, decentralization and interoperability, traceability in the supply chain was also identified as the main component, according to the assessment of the authors.

Table 1. Factors that most influence the competitiveness of the supply chain according to publications in the years 2018-2020.

Terms	No.	%	Main authors (> # of references in the review)
Traceability	393	26	Zhao *et al*., 2019; Helo and Shamsuzzoha, 2020; Sunny *et al*., 2020; Feng *et al*., 2020.
Supply Chain	265	17.5	Saberi *et al*., 2019; Kayikci *et al*., 2020; Helo and Shamsuzzoha, 2020.
Technological development	157	10.4	Torky and Hassanein, 2020; Zhang *et al*., 2020; Lu, 2018.
Trust	149	9.8	Ramírez, 2020; Mahyuni *et al*., 2020; Dave *et al*., 2019.
Data security	127	8.4	Qian *et al*, 2020; Li *et al*., 2018; Dutta *et al*., 2020; Zhao *et al*., 2019.
Competitiveness	117	7.7	Gloet *et al*., 2020.
Sustainability	111	7.3	Saberi *et al*., 2019; Kamble *et al*., 2020; Mahyuni *et al*., 2020.
Transparency	94	6.2	Casino *et al*., 2020; Dubey *et al*., 2020; Astill *et al*., 2019.
Decentralization	51	3.4	Varghese *et al*., 2019; Dutta *et al*., 2020; Lu, 2018.
Cost reduction	30	2.0	Gloet and Samson, 2020; Astill *et al*., 2019; Longo *et al*., 2019.
Interoperability	20	1.3	Lu, 2018; Saberi *et al*., 2019; Lezoche *et al*., 2020.
TOTAL	**1514**	**100**	

Table 2. Network of factors and associated codes to establish the strengths of blockchain implementation in agri-food innovation processes, review in the Period 2018- 2020.

Factor	No.	%
Traceability	393	47.1
Trust	149	17.9
Data security	127	15.2
Transparency	94	11.3
Decentralization	51	6.1
Interoperability	20	2.4
TOTAL	**834**	**100**

The research established two important lines to identify the strengths for the adoption of BT, traceability (47.1%) is the main element that is directly related to various monitoring processes and transmission of information from the origin of inputs to the sale or consumption of food, a tool that allows increasing the level of linkage of this system in the agri-food sector, the second network of codes is represented by the term trust (17.9%), establishing a high degree of association with data security processes, transparency, decentralization and interoperability terms, which can provide the appropriate level of credibility in each of the procedures of production, development, distribution and marketing of agri-food products to generate an easy adoption of blockchain technology.

Interaction between the levels of strength for BT implementation.

The interaction of the code network concerning the level of strength and its association for blockchain implementation in agri-food innovation processes can be observed in Table 3.

Ultimately, BT presents strengths where it increases conditions for tracking, recording, transmission and automated analysis of data by using a transparent, secure and intelligent network with the ability to communicate

Table 3. Strengths of blockchain implementation in agri-food innovation processes.

Main elements	Contribution in blockchain	Reference
Traceability	Improve yield by providing security and full transparency. Timely identify the source of food production.	(Feng, Wang, Duan, Zhang *et al.*, 2020).
	Develop an internal traceability system, improve traceability performance in food processing.	(Qian *et al.,* 2020).
	Ensure traceability and authenticity in the food supply chain.	(Galvez *et al.*, 2018).
	Improve traceability performance by providing full security and transparency.	(Astill *et al.*, 2019).
Trust	Reliable data storage with integrated privacy and management.	(Kayikci *et al.*, 2020).
	Its transparent, autonomous and secure nature can eliminate any possibility of manipulation, bias or error.	(Mahyuni *et al.*, 2020).
	Creates a bond of trust with users.	(Dave *et al.*, 2019).
Data security	Reducing the role of intermediaries in the network.	(Saberi *et al.*, 2019).
	Immutable data records, distributed storage and controlled user access.	(Mahyuni *et al.*, 2020).
	Ensuring data integrity and preventing tampering.	(Zhao *et al.*, 2019).
	Re-engineering business processes to improve security.	(Dutta *et al.*, 2020).
Transparency	Providing evidence of regulatory compliance to both state authorities and demanding customers.	(Casino *et al.*, 2020)
	Significant positive influence on the transparency of the operational supply chain.	(Dubey *et al.*, 2020)
	Increases the ability to track goods and reduces the need for a third party to monitor the network and control information.	(Ronaghi, 2020)
	Generates information security and integration of different stakeholders.	(Cardona and Orozco, 2019)
	Brand consumption increases when more information from the producer can be verified.	(Ramírez, 2020)
Decentralization	Create a new non-centralized programmable intelligent ecosystem.	(Lu, 2018)
	Minimize dependencies between organizations.	(Mahyuni *et al.*, 2020)
	Decentralized structure, distributed notes.	(Dutta *et al.*, 2020)
Interoperability	Agri-food 4.0, development of the sector based on digital technologies, as well as the process of interoperability between them.	(Lezoche *et al.*, 2020)

useful information in real-time to manage the supply chain (Dubey *et al.*, 2020; Ronaghi, 2020).

Influence of blockchain technology on supply chain competitiveness.

It is important to determine the conditions of greater influence of blockchain technology with greater economic and social profitability within an agri-food supply chain, considering that competitiveness is fundamental to automate traceability and transmission of information of productive and operational processes, with efficient and reliable security measures (Table 4).

Table 4 reports the conclusions of experts from the agri-food and technology sector who positively linked the adoption and application of blockchain technology in supply chain processes to the competitiveness factor (17.2%), the codes technological development (23.1%), sustainability (16.3%) and cost reduction (4.4%), which allowed to establish themselves as the main tools that could lead the company to stand out in the market, the supply chain code (39%), evidences the activity of blockchain in agri-food processes, it can be added that this relationship is growing in recent years due to the wide interest of professionals through scientific publications. In relation to the competitiveness that would be achieved with the application of blockchain technology, studies report that trust can be generated among stakeholders, which will allow them to inspect the record of the entire supply chain.

Table 4. Network of factors and associated codes to determine the influence of blockchain technology on the competitiveness of agri-food supply chains, review in the period 2018- 2020.

Factor	No.	%
Competitiveness	117	17.2
Technological development	157	23.1
Sustainability	111	16.3
Cost reduction	30	4.4
Supply chain	265	39
TOTAL	**680**	**100**

Stakeholders can comprehensively track information to determine the traceability and authenticity of each food product. In addition, BT can contribute significantly to sustainability (Galvez *et al*., 2018).

Also, a rapid evolution known as Industry 4.0 is taking place, comprising new digitization technologies such as blockchain and IoT that provide competitive advantages in supply chain users. The use of these new technologies is expected to improve process efficiency, speed and quality, which for perishable food products, in particular, is of great importance. Also, these technologies can improve product traceability and authenticity, which are valuable for domestic and export markets (Gloet and Samson, 2020).

When some external information or a sudden change in metrics occurs, management can target and investigate the next levels of metrics for detailed analysis. Acceleration of the decision-making cycle, through reliable real-time data, enables the growth of supply chain metrics, where speed in obtaining actionable information from data is a competitive feature. The control mechanism of performance metrics has remained similar: the loop has been accelerated, and data provide a valuable source of insight into the details and micromechanisms of operations (Helo and Shamsuzzoha, 2020).

Cost reduction. The adoption of traceability systems in the agri-food production chain by government institutions mainly covers legal and logistical aspects. The methodology is considered complex and costly, and nowadays they give greater consideration to the efficiency of logistical processes. Also, the importance of sanitary safety and the characteristics of the consumer market has increased (Ribeiro et al., 2020). The data and transactions carried out in the chain are recorded in the blockchain using smart contracts. The blockchain system is more efficient, more secure, more transparent and avoids intermediaries, resulting in lower costs for cooperative members, while generating greater confidence in distributors, supermarkets and consumers, developing a long-term benefit for small farmers and cooperatives (Borrero, 2019). A properly redesigned supply chain can achieve synchronization of tracking information across all business domains. In addition, the use of smart contracts can help reduce the time and costs required for supply chain reengineering (Dutta *et al*., 2020).

The food supply chain with IoT and blockchain-enabled applications can develop systems with increased cost-effectiveness, therefore the overhead associated with the technologies should be minimized and the resulting increase in transparency should translate into increased revenue for the company (Astill *et al*., 2019). The adoption of blockchain avoids the expenses of bank transfers, currency exchange, overhead and intermediation costs, it also reduces the use of paper in documents, certificates and printed reports (Pino and Prado, 2019). In addition, it promotes the efficient use of time and resource consumption to validate a transaction from days to seconds (Galvez *et al*., 2018; Avila, 2018).

Technological development. History has shown that technological advances that generate productivity gains prevail, blockchain technology will continue to be adopted throughout the global economy, shaping the future of that of agriculture, provided that productivity gains are real (Tripoli and Schmidhuber, 2018). Adoption of Industry 4.0 technologies is suggested as a strategy to establish agile processes in the supply chain ecosystem, seeking to meet dynamic demand, establishing collaborative networks and shared responsibility for a sustainable future (Sharma *et al*., 2020).

Intermediaries on the blockchain platform can provide basic functions without participating in the platform and service to both the producer and the consumer. The blockchain service provider helps its customers to integrate the platform with blockchain into their existing IT infrastructure and, in addition to consulting and implementation offers customization of the platform to meet specific customer requirements such as encryption, time chain and immutability (Tönnissen and Teuteberg, 2019). Industry 4.0 can generate the transformation towards a smart factory, when a fully articulated human-machine interaction is achieved, resolving the information asymmetry of technology, processes and collaborators. The complexity of this change lies not only in the lack of clear implementation guidelines in literature and structured information but also in enabling and convincing companies and operators about the advantages in their processes (Longo *et al*., 2019).

Sustainability. Agri-food supply chains play an essential role in achieving the UN Sustainable Development Goals, i.e., SDG 2 by ending hunger through achieving food security and improved nutrition and SDG 12 by ensuring sustainable consumption and production. Therefore, there is a need to investigate the impact of risks and build resilient agri-food supply chain organizations (Sharma *et al*., 2020).

The electronic sites used different technological tools to fulfill purposes such as promotion and awareness to the consumer about the importance of food selection, labeling, not throwing away leftover food but recycling it, making a new one. Helping small producers to reach, directly and without intermediaries, their products to consumers or a local market. Benefiting producers, as it lowers their costs, provides them with visibility and reduces poverty, as well as end buyers since they have the opportunity to follow the product they consume from harvest to sale (Porcelli, 2020). Blockchain technology can contribute to the sustainability of the social supply chain, distributing information in a stable and immutable way strengthening the sustainable building of the supply chain. Since information cannot be altered without the approval of accredited officials, blockchain can prevent corrupt users or organizations from illegal seizure of property. In addition, blockchain technology can block nefarious individuals and hold the dishonest accountable for both their social and individual misdeeds (Saberi *et al*., 2019).

Supply chain. The blockchain is formed through a series of connected blocks, where transaction history can be easily traced through previous blocks, making the technology transparent and reliable. Each block contains its own unique identification and has the hash of the previous block, ensuring secure transactions. All transactions are validated and recorded by the users of that network; they are also time-stamped, ordered chronologically are connected to the previous block and are irreversible once added to the network (Dutta *et al*., 2020). The current agri-food sector is transforming its integrated and centralized systems to shared and distributed systems. Most of the proposed frameworks to achieve higher performance are based on blockchain technology and cloud computing, which aim to provide secure, energy-efficient, and high-efficiency systems to agri-food manufacturers (Zhao *et al*., 2019).

Blockchain-based digitization and traceability can be used both in plants and in the animal food chain. However, the

Table 5. Influences of blockchain technology on the competitiveness of agri-food supply chains.

Main elements	Contribution of blockchain	Reference
Competitiveness	Great opportunity to establish automation.	(Lu, 2018).
	Collecting data from multiple stages within supply chains.	(Astill *et al.*, 2019).
	Traceability is the key to developing operational efficiencies and improving customer service.	(Varghese *et al.*, 2019).
	Track data and real-time location to generate key performance indicators.	(Dutta *et al.*, 2020).
	Minimize fraud and errors in supply chains, increase quality and safety of food products.	(Mirabelli and Solina, 2020).
	Greater autonomy and intelligence in managing precision agriculture in a more efficient and optimized way.	(Torky and Hassanein, 2020).
	Feed and pasture control, along with an increase in milk production and quality.	(Beltrán, 2020).
	In a trusted decentralized system, the consumer is willing to pay more for the same product.	(Ramírez, 2020).
Sustainability	Improved collaboration through rapid trust building among various stakeholders in operations.	(Dubey *et al.*, 2020).
	More social and transformative sustainability trends.	(Gloet and Samson, 2020).
	Decrease the need to transmit electricity over long distances.	(Mahyuni *et al.*, 2020).
	Improvements in production efficiency and reduction of resource and food waste.	(Astill *et al.*, 2019).
	Improved sustainable water management.	(Zhao *et al.*, 2019).
Cost reduction	The blockchain-based smart contract will have a big impact on transaction costs because the network executes it automatically.	(Mahyuni *et al.*, 2020).
	Saving time to make effective decisions based on objective data.	(Lezoche *et al.*, 2020).
	Elimination of middlemen to validate customer identification, information governance and transaction security.	(Pino and Prado, 2019).
Technological development	Deliver operational excellence by partnering with smart technologies.	(Zhang *et al.*, 2020).
	Introducing solutions for chronic safety and yield challenges in precision farming systems.	(Torky and Hassanein, 2020).
	Optimizing batch blending with AI, quality forecasting with big data and credible traceability with blockchain.	(Qian *et al.*, 2020).
	Facilitating the development of a distributed peer-to-peer network with high security, scalability and a well-structured cloud system.	(Li *et al.*, 2018).
	RFID (radio frequency identification) and IoT (Internet of Things) provide real-time information or data.	(Dutta *et al.*, 2020).
	Drive data-driven digital supply chain.	(Kamble *et al.*, 2020).
	Using sensors and drones, support data collection.	(Lezoche *et al.*, 2020).
	With distributed software architecture and advanced computing, can exchange information between chain players.	(Ronaghi, 2020).
	IoT and smart contract integration is dramatically elevating blockchain applications.	(Sunny *et al.*, 2020).
	IoT, big data analytics and visualization can help organizations achieve operational excellence in conducting life cycle assessment to improve supply chain sustainability.	(Zhang *et al.*, 2020).
Supply chain	Responsibility for distributing correct information.	(Kayikci *et al.*, 2020).
	Improve information exchange and flow of disaster relief funds.	(Dubey *et al.*, 2020).
	Store chemical analysis data in chronological order, so that it is impossible to manipulate later.	(Galvez *et al.*, 2018).
	Apply an algorithm to obtain a Food Quality Index (FQI).	(Varghese *et al.*, 2019).

challenges for such implementation are varied depending on different industry needs, workforce skill set and technical capabilities. But blockchain has great potential to save time, increase customer confidence, and reduce costs and risks in the food chain (Longo *et al*., 2019). Data in an agri-food supply chain are collected from all four supply chain processes, i.e., planning, sourcing, manufacturing, and delivery. However, the planning and delivery processes contribute greatly to the development of data analytics capability compared to the sourcing and manufacturing processes. The agri-food supply chain was found to use different resources for data collection and analysis (Kamble *et al*., 2020).

The significant benefits of this blockchain system in supply chain management can be increased distributed value, the satisfaction of a greater number of demands at the same time, improvements of input-output responses and customer cost can be easily boosted. It can be added that blockchain improves the visibility of supply chains, enabling automation of processes, eliminating middlemen and enabling real-time tracking through traceability, privacy and data management techniques, which are the cornerstones of supply chain reengineering (Dave *et al*., 2019).

In general, looking for strategies to highlight the agri-food sector companies in the global market, the participation of BT in production and operational processes can develop substantial improvements by integrating technological elements to ensure traceability procedures, allowing a sustainable development in business (Dutta *et al*., 2020; Sunny *et al*., 2020).

CONCLUSIONS

Blockchain technology is an innovative tool with the ability to create new profitable ecosystems with great support from society for economic and scientific development, it should be clarified that, in the last decade, growing economies stand out among other things for high investment in technology.

The review showed that a high level of integration of information and communication technologies aims to improve production and operational processes in the agri-food sector, streamline decision-making, production cycles and transport times, generating offers of innovative products in line with the real behavior of the market.

The study had several limitations, which can be addressed by future researchers, the research was focused on the agri-food supply chain, discarding pharmaceutical, maritime and unprocessed fresh food processes. Multiple production sectors should generate targeted initiatives with blockchain implementation to establish a competitive footprint in national companies and global markets.

The key to the relationship between blockchain and logistics is in the integration of their processes through sensors, automating documentation, incorporating real-time information and reducing the probability of human error, the studies reviewed highlight the strengths of this association generating importance in the elements such as traceability, trust, data security, transparency and decentralization as main features. This latter element becomes relevant in countries such as Colombia and Peru, where the agri-food chain is centralized.

State organizations responsible for the control of contaminated products could standardize surveillance concepts, developing computerized and proven processes of the supply chain in real-time, these procedures may have a high level of response and action in the prevention of foodborne diseases.

With the integrated involvement of the cloud, artificial intelligence, data analysis and the transmission of information in real-time using the Internet the accurate flow can be increased, enabling the authenticity and transparency of agri-food traceability systems. Determined that elements such as technological development, sustainability and cost reduction contribute to the quest to compete in globalized environments.

Finally, blockchain technology can improve supply chain sustainability by decreasing resource use, optimizing consumption and food quality, competitive companies aim to be efficient and scalable to address these issues in the long term. Operational staff training and knowledge development can be the key to success for early blockchain adoption, this training process should have a playful character to facilitate and increase the

number of collaborators that promote a positive impact on society.

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Review article

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Arbuscular mycorrhiza symbiosis in quinoa (*Chenopodium quinoa* Willd.): A systematic review

Simbiosis de micorriza arbuscular en quinua (*Chenopodium quinoa* Willd.): Una revisión sistemática

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ABSTRACT

The crop of quinoa has gained relevance during the last decade in different parts of the world, due to its adaptability to difficult edaphic and climatic conditions and the great nutritional potential of its seeds. However, climate change scenarios are increasingly adverse, so the search for strategies that favor greater adaptability of quinoa to areas where other crops fail to adapt is a scientific priority. For this reason, a systematic review was carried out, based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis methodology, with documents published on Scopus and Clarivate Web of Science databases. This methodology describes the diversity of fungi that favors symbiosis and the services offered by arbuscular mycorrhizal fungi in the physiological activity of the quinoa plant, in addition to their interaction with the edaphic conditions, mainly related to nitrogen and phosphorus. The results identified a projection of interest in research related to the symbiosis between these two organisms, but a very limited advance in relation to the study that has been developed around the microbiological activity of quinoa in the soil.

Keywords:
Cultivar
Diversity
Microorganisms
Nitrogen
Phosphorus
Plant physiology

RESUMEN

El cultivo de quinua ha tomado relevancia durante la última década en diferentes partes del mundo, debido a su adaptabilidad a condiciones edafoclimáticas difíciles y el gran potencial nutricional con el que cuentan sus semillas. Sin embargo, los escenarios del cambio climático cada vez son más adversos, por lo que la búsqueda de estrategias que favorezcan una mayor adaptabilidad de la quinua a zonas donde otros cultivos no logran adaptarse, es la prioridad científica. Por esta razón, se realizó una revisión sistemática, utilizando la metodología de elementos de informe preferidos para revisiones sistemáticas y meta-análisis. Se describe inicialmente la diversidad de hongos que favorecen la simbiosis y los servicios que ofrecen los hongos micorrízicos arbusculares en la actividad fisiológica de la planta de quinua, además de su interacción con las condiciones edáficas, principalmente relacionada con el nitrógeno y el fósforo. Los resultados identificaron una proyección de interés en la investigación relacionada con la simbiosis entre estos dos organismos pero un avance muy limitado en relación a estudio que se ha desarrollado en torno a la actividad microbiológica de la quinua en el suelo.

Palabras clave:
Cultivar
Diversidad
Microorganismos
Nitrógeno
Fósforo
Fisiología vegetal

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INTRODUCTION

Quinoa is a transient crop currently produced in several areas of the world, it is characterized by being adaptable to different edaphoclimatic conditions, resulting in region-specific quinoa cultivars (Bazile *et al*., 2016). These aspects bring about the concept "ecophysiology" defined as the discipline that explains the relationship between the physiological behavior of plants and the external conditions that affect their performance (Larcher, 2003), whereby aspects such as practices and interactions with their physical and biological environments contribute to the distinctive characteristics of quinoa grains grown in specific regions (García-Parra *et al*., 2020a).

In this sense, weather and soil characteristics, associated with the production area, have been studied in the last few years. Bosque-Sanchez *et al*. (2003) were the first scientists to talk about ecophysiology in quinoa. More recently, Murphy and Matanguihan (2015) considered the importance of seed characteristics of different quinoa cultivars, which was similar to that proposed by Reguera *et al*. (2018). Ruiz *et al*. (2014) highlighted the existence of edaphic microorganisms, capable of carrying out symbiotic relationships with different quinoa cultivars, however, despite the importance of these associations, their relevance and analysis have been scarce studied.

Among the great diversity of edaphic organisms, arbuscular mycorrhizal fungi are those with the greatest capacity to carry out symbiosis with quinoa roots (USDA, 2016). In this sense, this type of symbiosis is the most beneficial interaction between edaphic microorganisms and roots (Begum *et al*., 2019; Teste *et al*., 2020). The phylum Glomeromycota is the most important group of fungi in this activity, and therefore, it has been recognized that about 80% of terrestrial plant species including quinoa, manage to form a symbiosis with arbuscular mycorrhizae (Trouvelot *et al*., 2015), even though for many years, it was reported that some species belonging to the Chenopodiaceae and now Amaranthaceae family, it did not carry out edaphic symbiosis with fungi (Rydlová and Vosfitka, 2001; Chaudhry *et al*., 2005).

The mutualistic interaction between the mycorrhizal fungus and plant roots is based on the exchange of nutrients between these two actors, where the plant supplies carbon, while the fungus favors the activity of nutrient and water absorption, which takes place at the moment when some structures of the fungus colonize the cortical cells of the plant roots consolidating complex frameworks called arbuscules (Vierheilig, 2004; Janouskova *et al*., 2017). According to the above information, it becomes evident that this direct link between soil and quinoa allows to carry out a greater approach between the ecophysiological behavior of quinoa with its environment, which is important for the productive and scientific community of this species.

However, the high diversity of both fungi and cultivars of quinoa, as well as the edaphoclimatic conditions where the crop is established, make their relationship dynamic, increasing the interest of producers and research centers to know the characteristics and benefits of arbuscular mycorrhizae. For this reason, this work aimed to present a systematic review of the main aspects related to mycorrhizal activity between fungi and quinoa plants.

MATERIALS AND METHODS

A systematic review was carried out based on the PRISMA methodology (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) (Urrútia and Bonfill, 2010), with the research question: what is the relationship described by the scientific literature between fungi with mycorrhizal capacity and quinoa plants?

Search and data collection

To ensure the success and sensitivity of the search, the descriptor "soil", "quinoa" was consolidated using the Boolean operator AND in the conjugation “soil AND quinoa”. The exploration included all documents that find the search path in the title, abstract, and keywords initially; contemplating articles, books, book chapters, and conferences published in the Scopus and Clarivate Web of Science (WoS) databases following the methodology proposed by Yepes *et al*. (2018).

Selection criteria

Within the scientific papers obtained, a second filter was developed that based its selection on the following criteria: papers from journals were indexed in the Scimago Journal & Country Rank SJR database; papers published between 2000 and 2021 and that described theoretical aspects and scientific experiences of the activity of edaphic fungi with quinoa. In addition, special

importance was given to documents that included (i) diversity of edaphic fungi with mycorrhizal capacity in soils suitable for quinoa cultivation; (ii) importance of fungi that favor the nutrition of this species, and finally (iii) importance on the biological response of the species. All documents related to quinoa and that did not contemplate explanation, use or extermination with arbuscular mycorrhizal fungi were excluded.

Data processing and analysis

To determine the dynamics of the development of research related to soil AND quinoa, all the documents obtained under this search formula in the two databases were analyzed, taking the results from each of them and eliminating duplicates with the help of Mendeley reference manager. Subsequently, the documents found were organized in Excel® software by year, by the number of publications and the total. In addition, the main countries and research areas were detailed. Additionally, an analysis of publications was performed through non-linear regressions to determine their global trend, modeling the information in Sigmoidal 3-4-5 parameters, Logistic 3-4, Weibull 4-5 Gompertz 3, Hill 3-4 and Chapman 3-4 through the statistical program Sigmaplot (SystatSoftware Inc., San Jose USA). Data fit was selected through the coefficient of determination (R^2) and significance of the data (P<0.05) following the methodology used by García-Parra *et al*. (2020b).

RESULTS AND DISCUSSION

Through the analysis, it was determined that there are 239 documents published in the selected databases, distributed in research articles (205), review articles (11), book chapters (10), conferences (9), notes (2) and short review articles (1) between 2000 and 2020. In this context, the trend of research exposing the characteristics, use and management of soil, as well as its physical, chemical and microbiological properties with incidence on the biological performance of quinoa has grown exponentially, which is evidenced by the inflection point (2029) of the best fit model, which for this case was sigmoidal 3 parameters (R^2 0.997; P<0.05) (Table 1).

Table 1. Nonlinear regression models meeting the parameters for the search path "soil AND quinoa" analyzed since 2000.

Model	Inflection point	a	b	c	Value *p*	Durbin Watson	R^2
Sigmoidal 3	2029	1331.74	6.06	-	0.05	1.22	0.997
Hill 3	2044	462.13	92.45	-	0.80	0.06	0.395
Hill 4	2039	461.57	92.45	-18.37	0.90	0.06	0.427

The a, b, c, and inflection point values belong to the equation of the models used.

According to what was proposed by Escobar and Zartha (2017), the search areas of this systematic research are the key to incoming phase (Figure 1), which manifests an opportunity to produce this species, given as a consequence of the novel discovery of the symbiotic association between fungi and quinoa. In this sense, the search conjugation identified nine publications that met the criteria of the PRISMA methodology and that open in a specific way the relationship between quinoa plants and mycorrhizal activity, highlighting that quinoa has been recognized since 2013 as a promising crop against the effects of climate change, which is the reason why identifying the symbiotic association of this species with mycorrhizal fungi could enhance its production under more difficult edaphoclimatic conditions.

The regions with the greatest publication focus on quinoa and its relationship with the ecophysiological environment are developed countries, while developing countries such as those belonging to Latin Americana (Table 2) present contributions in a smaller proportion (García-Parra and Plazas-Leguizamón, 2019). In the case of Colombia, it has a participation of 0.83% in the world scientific production. In the case of Denmark, Chile, and Peru, the recognition in the study of quinoa is framed in the obtaining of the Plant Variety Certification (COV), whose

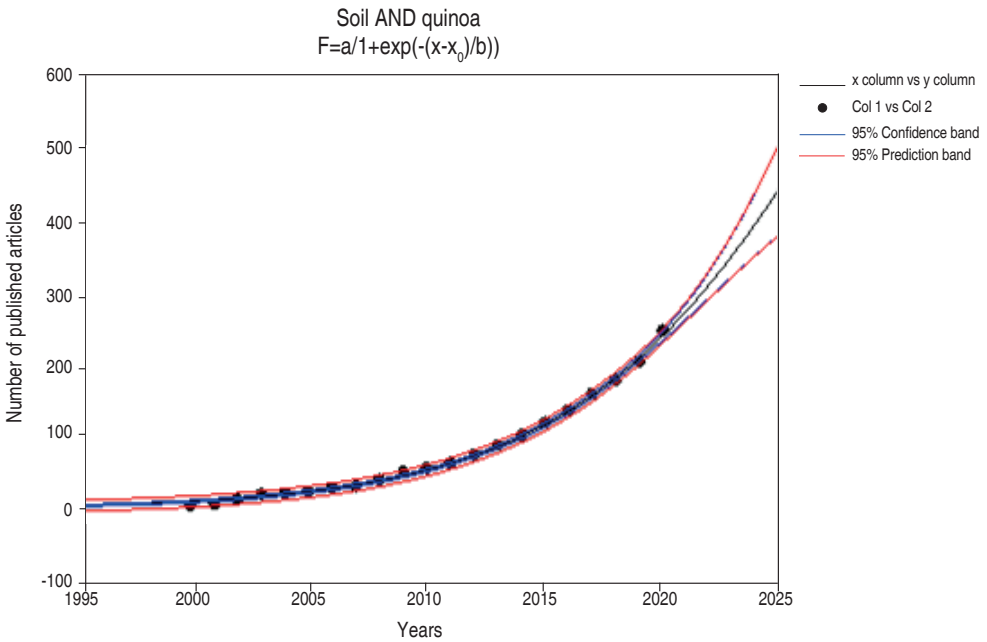

Figure 1. Nonlinear regression of the "soil AND quinoa" search path.

advantage lies in the genetic improvement activity and its in-depth study without having to refer to the country of origin (Bazile *et al*., 2014).

Given the interdisciplinarity involved in the production of quinoa crops, the development of publications addresses main areas such as agriculture and biological sciences (79.91%), environmental sciences (18.41%), biochemistry, genetics, and molecular biology (12.97%), engineering (5.85%), social sciences (5.02%) and earth and planetary sciences (4.6%), immunology and microbiology (4.6%), medicine (3.76), chemistry (2.51%), energy (2.51%) among others (11.29). The above demonstrates the growing interest that continues to develop the crop in aspects such as interactive dynamics with the soil, mainly, focused on the strategies used by the plant to capture water and nutrients (Choukr-Allah *et al*., 2016).

Table 2. Countries with the highest number of publications on the relationship between soil and quinoa (239 publications according to the Scopus database).

Highlighted countries	Percentage of publications (%)
Denmark	11.29
United States	10.46
China	9.62
Italy	8.36
Pakistan	8.36
Chile	7.94
Bolivia	5.85
Germany	5.85
Peru	5.85

It is relevant to highlight the nine publications that were most relevant according to the search for "soil AND quinoa" which contributed to the development of the discussion of the relationship between mycorrhizal fungi and their symbiosis with quinoa plants (Table 3).

Table 3. Most relevant publications according to the bibliometric search.

Publication	Authors	Journal	Year	Citations
Fungal root symbionts and their relationship with fine root proportion in native plants from the Bolivian Andean highlands above 3,700 m elevation	Urcelay, C. Acho, J. Joffre, R.	Mycorrhiza	2011	36
Root foraging capacity depends on root system architecture and ontogeny in seedlings of three Andean Chenopodium species	Alvarez-Flores, R. Winkel, T. Nguyen-Thi-Truc, A. Joffre, R.	Plant and Soil	2014	22
Distribution of arbuscular mycorrhizal fungi in upland field soil of Japan	Isobe, K. Aizawa, E. Iguchi, Y. Ishii, R.	Plant Production Science	2007	16
Quinoa: improvement and sustainable production	Murphy, K. Matanguihan, J.	Book – Wiley Blackwell	2015	14
Productivity and soil quality of organic forage, quinoa, and grain cropping systems in the dryland Pacific Northwest, USA	Wieme, R. Reganold, J. Crowder, D. Murphy, K. Carpenter-Boggs, L.	Agriculture, Ecosystems and Environment	2020	5
Distinct factors drive the assembly of quinoa-associated microbiomes along elevation	Cai, Z. Wang, X. Bhadra, S. Gao, Q.	Plant and Soil	2020	4
Effects of Inoculating Arbuscular Mycorrhizal Fungi on Growth of Quinoa under Different Phosphorus Levels	Chunhua, P. Shifang, Y. Yongqing, Z. Yanhong, H. Xiao, H. Yang, Y.	Crop	2017	4
Managing soil fertility and health for quinoa production and weed control in organic systems	Buckland, KR. Reeve, JR. Creech, JE. Durham, SL.	Soil & Tillage Research	2018	3
A Plant-Fungus Bioassay Supports the Classification of Quinoa (*Chenopodium quinoa* Willd.) as Inconsistently Mycorrhizal	Kellogg, JA. Reganold, J. Murphy, K. Carpenter-Boggs, L.	Microbial Ecology	2021	2

Consequently, the investigations listed in the Table 3 present the most important research advances in the world, where aspects related to fungal diversity are highlighted, its importance in the nutrition of the species, the transport of elements from the soil to the plant interior, as well as metabolic costs in symbiotic activity.

Importance of mycorrhizal diversity

The diversity of edaphic microorganisms has been studied mainly during the last decades, however, its study has been difficult due to the microscopic dimension of many of these species and the fact that many of them are hidden. In this sense, several factors affect their existence and their activity with other organisms, aspects that Landinez-Torres *et al*. (2019) recognize as determinants of fungal diversity, soil stability, and nutrient cycling.

All the fungi capable of establishing an arbuscular mycorrhizal symbiosis are grouped in the Glomeromycota phylum (Pedone-Bonfim *et al*., 2018); however, fungal families differ in their colonization strategy to the root, as demonstrated in Glomerales that carry out mycorrhization through hyphal fragments, while Diversisporales do it by spores, which is reflected in the colonization speed and the rapid adaptability of the plant to a particular stress condition (Trouvelot *et al*., 2015). According to this, differences in growth strategies between these fungi imply that soil management can greatly impact the diversity of arbuscular mycorrhizal fungi. For example, in quinoa, it has been reported that the greatest mycorrhizal formation occurs above 3700 masl and its fungal colonization dynamic is very similar to that of Poaceae (Urcelay *et al*., 2011).

Although research related to fungal diversity in quinoa is scarce, Cai *et al*. (2020) have reported a high presence of phylum such as Ascomycota, Basidiomycota, Zygomycota and Chydiomycota in the rhizospheric zone of quinoa plants, changing their percentage of abundance in relation to the altitude where the samples were collected. However, the authors recognize a high percentage of unidentified fungi that manage to carry out mycorrhization with this plant. This is an activity that is not only carried out in quinoa but also in other cultivated plants of agronomic interest such as maize (*Zea mays*), wheat (*Triticum aestivum*), and soybean (*Glycine max*), which naturally present a high diversity of mycorrhization (Renaut *et al*., 2020).

As it was discussed above, colonization of plant roots by edaphic fungi is an activity that occurs in many plants, and thus arbuscular mycorrhizal fungi (AMF) is not specific for a particular plant species. For example, Ascomycota is recognized for being the largest group of fungi capable of favoring nutrient uptake and generating a greater defense to pest and disease attack, while Basidiomycota has been recognized for its high colonization effectiveness in forested areas, where the presence of lignocellulosic material is abundant (Landinez-Torres *et al*., 2019). For the case of Zygomycota, its maximum performance has been manifested in soils where mechanical management and the use of external inputs is minimal, a habit that is very similar to the Chytridiomycota phylum (Panelli *et al*., 2017).

Thus, the question of AMF being associated with quinoa plants has been addressed in a few studies. This situation generated that the identification of the diversity of fungi capable of performing mycorrhization with this species will be carried out with detection methods based on DNA extraction and its amplification through PCR in three areas with different altitudes as reported by Cai *et al*. (2020). However, research has also analyzed the amount of spores present in rhizospheric soil, given that a relationship is found with the percentage of root colonization (Isobe *et al*., 2007).

This shows the importance of developing research related to the edaphic microbiota of quinoa, focused on showing the diversity of fungi, not only from the phylum but also from the species, since this would facilitate the knowledge of the real mutualistic activity that is developed between these two organisms and thus identify any advantage that strengthens the resistance of quinoa to soils with a higher saline activity, extreme water stress or strong temperature changes.

Nutritional physiology and its relationship with AMF

The physiological activity of plants is strongly related to the surrounding climatic conditions and the physical, chemical, and biological characteristics of the soil (Taiz and Zeiger, 2006). This is the reason why achieving favorable conditions for quinoa cultivation determines the success of seed production and grain quality (Reguera *et al*., 2018; García-Parra *et al*., 2019). Thus, carrying out fertilization plans for the plant to generate stimuli that favor its yield is complex, as it depends largely on the quinoa

cultivar to be used, soil characteristics, and even the type of production (foliage or grain). As a consequence of the multiple campaigns developed by different governmental and private entities regarding the adaptability of quinoa to areas where other crops cannot survive, most quinoa production systems are located in soils with low availability of nutrients, water, conditions of salinity and strong temperature changes; scenarios that in many cases generate changes at the physiological, phenological and morphological level of quinoa (Ruiz *et al*., 2014).

It is well known that a good AMF colonization occurs under conditions of low nutrient availability in plants or soil (Begum *et al*., 2019), where fungi colonize the root tissue of the plant and seek a greater exploration of soil area, to absorb nutrients and water, which are transported to the interior of the plant through the hyphal networks. However, this activity generates a physiological cost, which translates into a constant demand for carbon energy sources that facilitate the metabolic activity of the fungus; this interaction is estimated to demand between 10 and 30% of the photoassimilates produced by the plant, used for the formation, maintenance, and functionality of the mycorrhizal structure (Alarcón and Ferrera-Cerrato, 1999).

Nevertheless, all this biological activity is also determined by the root architecture of the plant (Bender *et al*., 2014). The root architecture of quinoa is recognized for being highly branched and with good development in the density of fine roots, which favors the ability to colonize the soil and increase the uptake of water and nutrients that are translocated by the plant and transported into biomass and seeds with greater efficiency in this species compared to others belonging to the *Chenopodium* subfamily (Alvarez-Flores *et al*., 2014). Therefore, once plants are colonized by AMF, nutrient uptake efficiency increases since nutrient uptake can occur through the root or the arbuscular mycorrhiza involving the hyphal structure of the fungus, this latter with a greater facility to explore areas where the root cannot reach (Liu *et al*., 2016).

Phosphorus

A morphological character of AMF in the plant is its penetration into the cortical cells of the root and the development of a prominent hyphal structure. However, this aspect is highly variable among plants, fungal groups, and edaphoclimatic characteristics (Kobae, 2019). In this sense, the theory that most accurately explains the interaction of these dynamics is the one that proposes that although AMF rapidly colonizes the interior of the root cell, the arbuscular structure does not completely fuse with the colonized cell, there is also no instantaneous flowing exchange, given that the fungal structure is surrounded by periarbuscular membranes that can change in thickness and composition according to the type of fungus, determining the rate of exchange of substances by the two organisms (Kobae and Hata, 2010; Camarena-Gutierrez, 2012).

It is well known that phosphorus is a vital element in plant physiology since this element is a structural part of genetic chains, metabolic energy, and accessory structures of cell membranes mainly (Shen *et al*., 2011). Nonetheless, plants encounter different difficulties when taking it from the soil, mainly due to its low mobility, the effect of acid pH, soil colloidal dynamics, and microbial activity (Marschner, 2012).

Consequently, the increase of arbuscular structures in the root tissue favors the expression of genes that facilitate the capture of phosphates from the fungal structure to the plant tissue, through proteins located in the periarbuscular membrane (Harrison *et al.,* 2002). However, the dynamics developed for the exchange of elements such as phosphorus between the plant and the fungus are variable. This is because active colonization presents an accelerated movement of phosphorus, mainly when it is scarce. Furthermore, senescent colonization occurs when the hyphal network stops growing and, therefore, it is still unknown whether phosphorus mobility continues between the plant tissue and the arbuscular branch (Kobae *et al*., 2016) (Figure 2).

In quinoa plants, the combined effect of the application of edaphic phosphorus and AMF (*Glomus mosseae* and *Glomus tortuosum*) on physiological parameters has been studied, resulting in a significant increase in vegetative growth, chlorophyll content, photosystem II photochemical efficiency (Fv/Fm) and photosystem II potential activity (Fv/Fo) during the initial phase of the trial, followed by a decrease in physiological vigor after the application of the phosphorus treatments compared to the treatment without AMF inoculation; It was also found that quinoa plants with AMF application showed better physiological parameters except for root diameter (Chunhua *et al*., 2017).

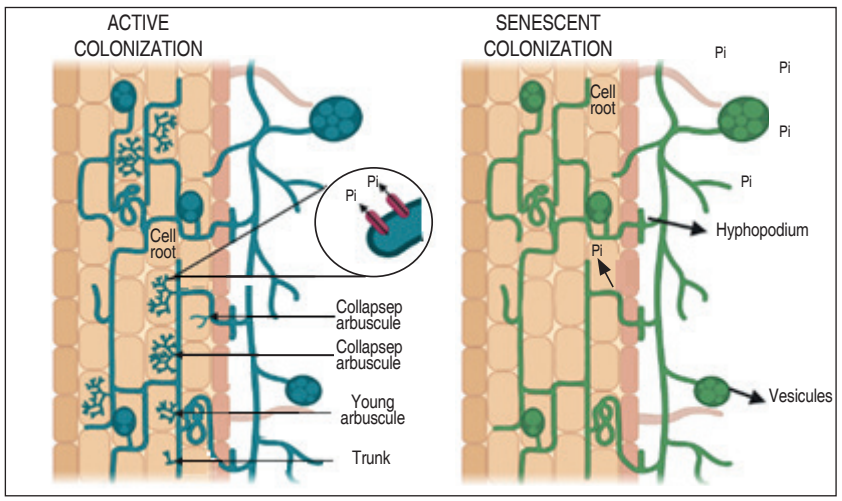

Figure 2. Colonization dynamics of fungi with mycorrhizal capacity.

According to different scientific antecedents, it is possible to indicate that the benefits offered by AMF to the host plant, demand a carbon cost, which are normally attributed to carbohydrate and lipid compounds product of the photosynthetic activity of the plant and that serve the fungus to support the growth of different structures such as hyphae and spores mainly (Keymer *et al*., 2017), which maintains the mutualistic activity of the organisms through a molecular dialogue between the plant and AMF, including complex networks of perception and signal transduction of genes from both symbiotic partners (Panelli *et al*., 2017). For the case of plants, bioactive signals are mainly given by strigolactones and (iso) flavonoids, while AMF secretes lipochitooligosaccharide (LCO) and short-chain chitin oligomer (Nanjareddy *et al*., 2017). At present, few studies have reported the relationship of symbiotic activity between fungi and quinoa, so more efforts are needed to investigate the mechanism that develops between these two organisms, mainly in the activity with phosphorus uptake.

Nitrogen

As a consequence of the high demand for nitrogen by the quinoa crop, its capture becomes crucial for the development of metabolism related to protein structuring and in the development of photosynthetic activity in this species (García-Parra *et al*., 2019). Thus, nitrogen is considered an essential element, given that it determines the phenological cycle of quinoa and the protein potential of its seeds, which is significantly higher compared to cereals (Bascuñán-Godoy *et al*., 2018b) but is strongly influenced by the availability and absorption of nitrogen in relation to soil type and organic matter turnover and, therefore, this characteristic is highly changeable between edaphic microclimates.

Although nutritional interactions related to nitrogen in quinoa cultivation, have been widely studied (González *et al*., 2009; Bascuñán-Godoy *et al*., 2018a; Bascuñán-Godoy *et al*., 2018b), quinoa production systems do not optimize the supply of this element and, on the contrary, make excessive use of nitrogen fertilizers. Because of its importance, AMF has established a relevant relationship with plants, no greater than that developed around phosphorus. AMF can take up nitrogen in the form of NO_3^-, NH_4^+ and as organic nitrogen, however, the fungus presents a strong preference for NH_4^+, which is the most assimilable form and of lower energy expenditure by the plant after reducing NO_3^- into NO_2^- by enzymatic action and this transformed into NH_4^+ by the effect of nitrite reductase (Fonseca-López *et al*., 2020). AMF colonization in quinoa has been studied over time and has been compared with other food crops such as wheat (*Triticum vulgare*), barley (*Hordeum vulgare*) and chickpea (*Cicer arietinum*), showing a low percentage of colonization in relation to the content of hyphae, vesicles, and arbuscules as was reported by Wieme *et al*. (2020), which determines that further study of the response of this species to AMF colonization and its relationship with nutrient transport should be consolidated.

In addition to the application and uptake of inorganic nitrogen, it has been established that AMF absorbs substantial amounts of organic nitrogen (Trouvelot *et al*., 2015) and that by the nature of the soil and climatic conditions such as temperature, its uptake into the plants is favored. In fact, in organic quinoa production systems the incorporation of behaved animal excreta and harvest residues has been used to increase the presence of nitrogen in the soil, expressing favorable results (Buckland *et al*., 2018; García-Parra *et al*., 2019), in benefit of the speed of mineralization of the material and the activity of organisms such as AMF and bacteria mainly.

Carbon cost due to the effect of symbiosis

Carbon demand by AMF is a considerable aspect for the colonized plant, as the fungus can receive between 4 and 20% of carbon photosynthetically fixed by the plant (Soudzilovskaia *et al*., 2019). Quinoa, as a transient plant, depends on an accelerated mobility of C during its productive cycle, so an imbalance during its development, would affect productivity, given that the low amount of reserves that it manages to accumulate for long periods in the leaves. AMF growth and nutrient acquisition depend on plant reserves, therefore, the mobility of C reserves and plant development could be significantly affected, as reported by Kellogg *et al*. (2021), who evaluated different quinoa cultivars that were subjected to mycorrhization and showed an increase in dry biomass production, while there was negative growth in plant height compared to the control treatment; an aspect in which the authors emphasized the importance of not colonizing with AMF in this species, given the heterogeneity that quinoa shows when colonized with these organisms.

However, the biochemical activity that is developed around the symbiosis between AMF and quinoa is very uncertain, so research is needed to detail its effect in relation to their interaction, in terms of the great diversity of fungi that can colonize and the wide diversity of quinoa cultivars.

AMF and water stress

The biological state of the soil and the water use of the plants play a key role in quinoa physiological behavior and grain quality. Nevertheless, given the campaigns developed by different governmental and private entities, the adaptability of quinoa in marginal areas, with problems of salinity, water availability, and nutritional deficit, this species has had to generate different metabolic strategies that allow its normal development. In this sense, it has been shown that climate change affects the phenological development of quinoa (Jaikishun *et al*., 2019; García-Parra *et al*., 2020a) and despite this, quinoa generates strategies for its adaptability as demonstrated Reguera *et al*. (2018), who found a differentiated behavior of quinoa, under different agroecological zones of production. Thus, the agronomic and management strategies of quinoa crops play a determining role in supplying water and nutrients to plants and, therefore, it is known that AMF can absorb and transport water and nutrients into the plant tissue, thus, an increase in the extension of fungal mycelium can be decisive in dry seasons.

This situation, in which the fungus manages to extend its mycelium, favors soil structuring, since the development of complex and branched networks can bring soil particles together and improve their structure (Rillig and Mummey, 2006), which together with the secretion of mucilages, polysaccharides and extracellular compounds such as glomalin and hydrophobins can increase the hydrophobic organic matter that creates more stable aggregates in the face of changes in soil water status (Rashid *et al*., 2016) and that would favor joint activity with organisms such as bacteria, which act freely in quinoa, and that would favor the availability of more nutrients for this crop and the retention of water in soil colloidal matrices.

Nevertheless, despite the knowledge gap regarding the symbiotic interaction between quinoa plants and AMF, it is possible to intuit that quinoa plants do not fully recognize AMF as a beneficial agent and, therefore, synthesize secondary metabolites such as phytoalexins, which have been found in high contents in quinoa and are capable of generating control by the attack of pathogenic fungi and pests (Yactayo-Chang *et al*., 2020).

Experiences of AMF in quinoa

Through the growing study of quinoa during the last decades, research has allowed the establishment of some scientific experiences that highlight the relationship between AMF and quinoa. In summary, this section will highlight the most relevant trials that

relate to the mutualistic interaction of the two organisms mentioned above. In this sense, the search analysis determined that the research related to this topic has been developed basically in the United States, China, Japan, Finland, and Bolivia, while the diversity of fungi found in association with this plant is scarce (Table 4).

Table 4. Application of mycorrhizal fungi in different countries.

Country	Cultivar	m a.s.l.	HMF	Structures	Source
United States	Rosa Junín	787	*Funneliformis mosseae*	Hyphae	Kellogg *et al*. (2021)
China	-	450	*Glomus mosseae* *Glomus tortuosum*	Hyphae	Chunhua *et al*. (2017)
United States	KU-2	779	-	Hyphae, vesicles and arbuscules	Wieme *et al*. (2020)
Japan	Yellow from Marangani	30	-	-	Isobe *et al*. (2007)
China	White and red	1100	Ascomycota, Basidiomycota, Zygomycota y Chydiomycota	Hyphae	Cai *et al*. (2020)
Finland	-	134	*Glomus* spp.	-	Vestberg *et al*. (2012)
Bolivia		3700	*Olpidium*	Hyphae, vesicles and arbuscules	Urcelay *et al*. (2011)

Wieme *et al*. (2020) reported that the symbiotic association between quinoa and fungi developed through the formation of structures such as hyphae, vesicles, and arbuscules, although their presence is significantly lower compared to other crops of food interest such as wheat, chickpea, and barley. Additionally, Urcelay *et al*. (2011) identified abundant radical colonization of *Olpidium*, with an abundant presence of mycelium, vesicles, and arbuscules, however, this fungus is recognized for its pathogenic capacity, which draws the attention of researchers and suggests the importance of carrying out trials around the activity of these two organisms.

CONCLUSIONS

Quinoa presents a response to the presence and colonization of fungi with mycorrhizal capacity, which can manifest itself as a beneficial agent, capable of developing symbiotic activity, or generate physiological and biochemical responses that recognize this organism as a pathogenic agent. Because of the uncertainty expressed by quinoa in the presence of fungi with mycorrhizal capacity, the references highlight the need to study the behavior that different quinoa cultivars may show when applied under controlled and field conditions. In general, it has been reported that the major fungal structure found inside the root cells of quinoa is mycelium, which does not necessarily indicate that there is a constant activity of exchange of substances that benefit both organisms.

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Short article

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Isolation of a *Leuconostoc mesenteroides* ssp. *jonggajibkimchii* strain from *Parona leatherjacket* (*Parona signata*): behavior in vegetal matrices fermentation

UNIVERSIDAD NACIONAL DE COLOMBIA

Aislamiento de una cepa de *Leuconostoc mesenteroides* ssp. *jonggajibkimchii* de palometa de mar (*Parona signata*): comportamiento en la fermentación de matrices vegetales

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Keywords:
Brassicaceae
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ABSTRACT

A Gram-positive, facultatively anaerobic, gas-forming, catalase-negative, nonmotile, non-spore-forming, vancomycin-resistant, and ovoid-shaped bacterium, designated strain Tw234, was isolated from the intestinal tract of Parona leatherjacket (*Parona signata*). The strain grew in the presence of 0–6% (w/v) NaCl, at pH 3.5–8.5 and 8–40 °C; optimum growth was achieved at 1% (w/v) NaCl, at pH 6.0 and 30–32 °C. Exopolysacharides production was detected by the solidification test of skim milk supplemented with sucrose in the temperature range of 8 to 30 °C. Results of phylogenetic analysis based on the 16S rRNA gene sequence similarity indicated that strain Tw234 was closed related to the genus *Leuconostoc* and 100% homology with the type strain *Ln. mesenteroides* ssp. *jonggajibkimchii* DRC1506 (KCCM 43249, JCM 31787). The evaluation of growth and acidification rates were carried out in white cabbage and Chinese cabbage and compared with the strain *Ln. mesenteroides* ssp. *jonggajibkimchii* RCTw1.1, isolated from the spontaneous fermentation of red cabbage. No significant differences were observed between the behaviors of the two strains. The strain Tw234 displayed higher growth and acidification rates in controlled fermentation of white cabbage compared with those obtained in Chinese cabbage. New trends are targeted on the isolation and selection of strains to achieve controlled fermentation of vegetables that may ensure uniform quality. The results obtained in this work suggest that strain Tw234 harbored technological useful properties for its potential use as a starter in controlled vegetable fermentations.

Palabras clave:
Brassicaceae
Fermentación controlada
Cultivo iniciador

RESUMEN

Una bacteria Gram-positiva, anaeróbica facultativa, formadora de gas, catalasa negativa, no móvil, no formadora de esporas, resistente a la vancomicina y de forma ovoide, designada cepa Tw234, se aisló del tracto intestinal de palometa de mar (*Parona signata*). La cepa creció en presencia de 0–6% (p/v) NaCl, a pH 3.5–8.5, 8–40 °C; el crecimiento óptimo se logró con 1% (p/v) de NaCl, a pH 6.0, 30–32 °C. La producción de exopolisacáridos se detectó mediante la prueba de solidificación de la leche descremada suplementada con sacarosa en el rango de temperatura de 8 a 30 °C. Los resultados del análisis filogenético basado en la similitud de la secuencia del gen ARNr 16S indicaron que la cepa Tw234 se relaciona con el género *Leuconostoc* y muestra un 100% de homología con la cepa tipo *Ln. mesenteroides* ssp. *jonggajibkimchii* DRC1506 (KCCM 43249, JCM 31787). La evaluación de las tasas de crecimiento y acidificación se realizaron en repollo blanco y repollo chino, y se compararon con la cepa *Ln. mesenteroides* ssp. *jonggajibkimchii* RCTw1.1, aislada de la fermentación espontánea de repollo rojo. No se observaron diferencias significativas entre los comportamientos de las dos cepas. La cepa Tw234 mostró mayores tasas de crecimiento y acidificación en la fermentación controlada de repollo blanco en comparación con las obtenidas en repollo chino. Las nuevas tendencias se dirigen al aislamiento y selección de cepas para lograr una fermentación controlada de vegetales que asegure una calidad uniforme. Los resultados obtenidos en este trabajo sugieren que la cepa Tw234 posee propiedades tecnológicas para su potencial uso como “starter” en procesos controlados.

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The interest in plant fermentations has increased over time, since they constitute a mode of conservation that avoids the use of chemical additives or the implementation of preservation methods that may alter the physical and organoleptic characteristics of treated foods (Di Cagno *et al*., 2013). Brasicaceous or cruciferous have been used since ancient times in spontaneous fermentations and have become a model that allows the study of changes that occur during the process (Fusari *et al*., 2020; Seong *et al*., 2016). Spontaneous fermentations occur due to the action of natural epiphytic biota, mainly lactic acid bacteria (LAB). Plant biotype, region, climatic conditions, and growing conditions (Parkash *et al*., 2017; Tanyi *et al*., 2018) influence the quality of the biota, which makes it difficult to control the fermentation process and maintain the uniformity of the final product (Capozzi *et al*., 2017). In recent years, to avoid this drawback, the use of starters as a way to control the bioprocess has been proposed by several researchers (Johanningsmeier *et al*., 2007; Lillo-Pérez *et al*., 2021; Montemurro *et al*., 2021).

Several companies market products that contain selected LAB strains based on their biotechnology properties; however, they are not fated for specific vegetables or fruits (Di Cagno *et al*., 2013). Some food companies linked to the marketing of fermented vegetables have taken the decision to design and use native starters. This process involves the isolation and selection of strains with specific metabolic characteristics from a selected plant, for later use, under controlled fermentation conditions on the same substrate (Gu *et al*., 2012; Kim *et al*., 2019; Lee *et al*., 2020).

Kimchi, a traditional food of Korean culinary culture is the most cited and studied example in the use of native starters in controlled vegetable fermentations. Homemade kimchi is elaborated based on the spontaneous fermentation of Chinese cabbage where other vegetables such as radish, garlic, leek, pepper, and ginger are added (Jung *et al*., 2014).

In recent years its consumption increased worldwide, not only due to its organoleptic characteristics but also because of the beneficial effects on consumer health (Özer and Yıldırım, 2019). This phenomenon has increased the interest of Korean state authorities and food industries in introducing standardized protocols that include indigenous ferments to avoid the random quality of spontaneous fermentations (Kang and Lee, 2020). The main genera, among LAB, responsible for kimchi fermentation are the *Leuconostoc*, *Lactiplantibacillus*, and *Weissella* (Di Cagno *et al*., 2013; Jung *et al*., 2014). Therefore, these genera have been intensely studied to achieve a greater understanding of the influence it exerts on the quality of the product and the nutritional benefits. Among genus *Leuconostoc*, several species have been isolated from kimchi; some of them are marketed under trademarks as specific starters for the fermentation of this traditional Korean food. *Leuconostoc miyukkimchii*, *Ln. kimchii* and subspecies of *Ln. mesenteroides* have been used with success on an industrial scale (Chun *et al*., 2017; Lee *et al*., 2012; Oh *et al*., 2010).

Ln. mesenteroides ssp. *jonggajibkimchii* has been accepted as new subspecies since 2017, after the analysis of its complete genome sequence, and is currently marketed through Daesang Company, the patent owner (Jeon *et al*., 2017). The strain is recommended as a starter in kimchi elaboration due to the development of significantly excellent sensory properties compared to spontaneous fermentation. It is acid-resistant and synthesizes significant mannitol concentrations, a compound that increases the refreshing taste.

This work reports the first isolation of a *Ln. mesenteroides* ssp. *jonggajibkimchii* strain from the intestinal content Parona leatherjacket (*Parona signata*). The biochemical profile was compared with the type strains *Ln. mesenteroides* ssp. *jonggajibkimchii* DRC1506 and *Ln. mesenteroides* ssp. *suionicum* DSM20241. Exopolysaccharides production and the influence of temperature, salinity, and pH on bacterial growth were also studied. The evolution of bacterial population and pH was monitored in controlled fermentation of white cabbage and Chinese cabbage, and compared with the results obtained with *Ln. mesenteroides* ssp. *jonggajibkimchii* RCTw1.1, a strain isolated from the spontaneous fermentation of red cabbage.

MATERIALS AND METHODS

Bacterial strains

The bacterial strain Tw234 was isolated from the intestinal content of Parona leatherjacket (*Parona*

signata), collected in the Port Rawson bay, Chubut, Argentina (latitude -43.30016; longitude -65.10228). The strain *Ln. mesenteroides* ssp. *jonggajibkimchii* RCTw1.1 (GenBank accession numbers: MT702992) belonging to Laboratorio de Biotecnología Bacteriana (Facultad de Ciencias Naturales y Ciencias de la Salud, Universidad Nacional de la Patagonia San Juan Bosco, Trelew) was used for comparative studies.

Phenotypic identification

The strain Tw234 was subjected to the following biochemical tests: Gram stain, catalase activity, oxidase activity, and gas production (CO_2) from glucose (Björkroth and Holzapfel, 2006). The carbohydrate fermentation patterns were studied using the API 50 CHL system (BioMérieux, Lyon, France), following the manufacturer´s recommendations.

Influence of temperature, pH and salinity on growth

The growth of the Tw234 strain was evaluated against different conditions of temperature, pH, and salinity. The growth was determined in Man Rogosa Sharpe broth (MRS) (Biokar, France) during 48 h of incubation at different temperatures (8, 10, 15, 20, 25, 30, 37, 40 and 45 °C) and pH values (3.0 to 9.0, at intervals of 0.5 pH units). Tolerance to NaCl was determined in MRS broth using concentrations between 1 and 8%, after 48 h of incubation at 30 °C.

Exopolysaccharides production

Exopolysaccharide (EPS) production was assessed on Brain Heart agar (Biokar, France) supplemented with Congo Red 0.8 g L^{-1} and sucrose 50 g L^{-1} (Freeman *et al*., 1989). The plates were incubated at 18 °C for 5 days. Exopolysaccharide production is detected by the black color development of colonies in the medium.

The solidification of fermentation milk supplemented with 3, 6, 9, and 12% (w/v) sucrose was used as a complementary assay (Wang *et al*., 2019). Skim milk without sucrose was used as a control. Incubation was carried out at 30 °C for 48 h and 8 and 10 °C for 5 days.

Genotypic identification

The strain was incubated in MRS broth at 30 °C for 18 h and was centrifuged at 12,000 *g* at 4 °C for 5 min; the total genomic DNA was extracted using Wizard Genomics kits (Promega, Madison, Wisconsin, USA) following the manufacturer's instructions. For identification of isolated strain, two universal primer pairs, 27F (5´-AGAGTTTGATCCTGGCTCAG-3´) and 1492R (5´-GGTTACCTTGTTACGACTT-3´) were used for amplifying the 16S rRNA gene in a Multigene Gradient thermal cycler (Labnet International Inc., USA). The amplified DNA was sequenced using the Macrogen sequencing service (Macrogen Inc., Seoul, Korea). The 16S rRNA gene sequence was compared with 16S rRNA gene sequences in NCBI's GenBank using BLAST (Basic Local Alignment Search Tool https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul *et al*., 1990).

Phylogenetic analysis

The phylogenetic tree was constructed using the Neighbor-Joining method (Saitou and Nei, 1987) with software package MEGA version 6.0 (Tamura *et al*., 2013). Tamura-Nei substitution was the model used, with a bootstrap value of 1,000 replicates. The resultant 16S rDNA sequence of the isolate was aligned against representative sequences of collection strain obtained from the database Ribosomal Database Project (RDP) and the National Center for Biotechnology Information (NCBI). The *Weissela confusa* sequence was chosen as an outgroup strain due to its phylogenetic relationship with the *Leuconostoc* genus.

Plant material and fermentation process

The Chinese cabbage (*Brassica rapa* L. var. *glabra*, Regel) and white cabbage (*B. oleracea* L. ssp. *capitata*, Metzg.) were obtained from the farm of the Valle Inferior del Río Chubut, Patagonia Argentina (latitude -43.14, longitude -65.19, 11 masl). Before the preparation of vegetables for fermentation, dry outer leaves of the first group of bulbs were removed. The cleaned bulbs were chopped in a shredder into 2 mm thick strips and supplemented with NaCl 3% (w/w). Shredded cabbages were autoclaved at 121 °C for 3 min.

The strains Tw234 and *Ln. mesenteroides* ssp. *jonggajibkimchii* RCTw1.1 were individually used as starters, according to the method described by Xiong *et al*. (2014) with some modifications. The strains were cultivated in MRS broth for 18 h at 30 °C to reach a concentration of 10^9 CFU mL^{-1}, later were centrifuged (4,000 *g*, 15 min). The cell pellets were washed twice

and resuspended in distilled water. The vegetables were individually inoculated with the starters reaching a final population of approximately 10^4 CFU g^{-1} and incubated at 18 °C for 96 h.

Fermentation parameters

The pH and LAB growth were monitored at 0, 2, 12, 18, 24, 36, 48, 72, and 96 h. The pH determination was carried out with a calibrated pH-meter (Orion 410). The growth was monitored on MRS agar using serial dilutions of sauerkraut brine samples; the plaques were incubated at 30 °C for 48 h (Lanza *et al*., 2020). The growth data of the strains RCTw1.1 and Tw234 obtained during the controlled fermentation were fitted using the Gompertz equation (1):

$$\ln\left(\frac{N_t}{N_0}\right) = A \times \exp\left\{-\exp\times\left[1+\frac{\mu_m}{A}(\lambda - t)\right]\right\} \qquad (1)$$

Where N_t is the number of microorganisms (CFU mL^{-1}) at time t (h), N_0 is the number of microorganisms at the inoculation time. Bacterial growth parameters are: A asymptotic value, μ_m the maximum specific growth rate (h^{-1}), and λ the lag time (h) (Biesta-Peters *et al*., 2010). The experimental data were fitted by nonlinear regression using STATISTICA software (Statsoft, Tulsa, Oklahoma, USA). The coefficient of determination (R^2_{adj}) and root mean square error (RMSE) were used as criteria for adequacy of fit.

Statistical analysis

The fermentations were carried out in duplicate. The results were subject to a one-way analysis of variance (ANOVA). The pairs of means of the treatments were compared applying the Tukey test ($P \leq 0.05$), using the statistical package STATISTICA software (Statsoft, Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

The general biochemical characteristics exhibited by the Tw234 strain correspond to those described for the genus *Leuconostoc*: catalase-negative, oxidase-negative, gram-positive cocci arranged in pairs or short chains, heterofermentative and vancomycin-resistant (30 µg mL^{-1}) (Chun *et al*., 2017).

The growth on MRS broth of the Tw234 strain was observed at temperatures between 8 and 40 °C, with an optimum growth temperature between 30–32 °C, but no growth was observed below 8 and at 45 °C. At 8 °C there was a remarkable difference compared with the collection strain *Ln. mesenteroides* ssp. *jonggajibkimchii* DRC1506, which does not exhibit growth at temperatures below 10 °C according to its description (Jeon *et al*., 2017). The strain *Ln. mesenteroides* ssp. *jonggajibkimchii* RCTw1.1, belonging to our collection and isolated from spontaneous fermentation of red cabbage, exhibited growth at 8 °C similar to the studied strain. This metabolic feature, perhaps derived from the selective pressure exerted by the Patagonian marine environment's low temperatures, has potential application in the food industry. For instance, the organoleptic characteristics of kimchi depend on the processing temperature and have been demonstrated to be superior when the fermentation process occurs at temperatures below 10 °C. Tw234 y RCTw1.1 strains displayed growth between 3.5–8.5 pH values while the type strain DRC1506, according to its description, showed a growth pH range of 4.0–9.0. NaCl tolerance values of Tw234 and RCTw1.1 strains were recorded in concentrations from 0 to 6%, as those values reported for the strain type DRC1506 (Jeon *et al*., 2017).

Exopolysaccharides have varied applications in different industries as they can be used as gelling agents, bio-flocculants, stabilizers, or emulsifiers. In the particular case of food, its production during fermentation increases viscosity and improves the final texture of the product (Wang *et al*., 2019). The Tw234 strain was able to produce exopolysaccharides at 30, 10, and 8 °C, when the test was conducted in BHI agar, supplemented with sucrose and Congo red. Exopolysaccharides production was also detected when the milk solidification test was performed on all sucrose concentrations assayed. Solidification was achieved after 48 h of incubation when the test was performed at 30 °C, while the same effect was detected, after five days, when the test was carried out at 8 °C.

In Table 1 can be observed the fermentation profile of the Tw234 strain, *Ln. mesenteroides* ssp. *jonggajibkimchii* DRC1506, *Ln. mesenteroides* ssp. *jonggajibkimchii* RCTw1.1, and *Ln.mesenteroides* ssp. *suionicum* DSM 20241. The latter strains were included because they are phylogenetically very close to *Ln. mesenteroides* ssp. *jonggajibkimchii,* and were reclassified as representing two different subspecies in 2017, after the complete sequencing of both genomes (Gu *et al*., 2012; Jeon *et al*., 2017).

Table 1: Biochemical identification with API 50 CHL system.

Sugars	Strains			
	Tw234	RCTw1.1	DRC1506	DSM20241
Glycerol	-	-	-	-
Erythritol	-	-	-	-
D-Arabinose	-	-	-	-
L-Arabinose	+	+	+	+
D-Ribose	+	+	+	+
D-Xylose	-	-	+	+
L-Xylose	-	-	-	-
D-Adonitol	-	-	-	-
Methyl-b-D-Xylopiranoside	-	-	-	-
D-Galactose	+	+	+	+
D-Glucose	+	+	+	+
D-Fructose	+	+	+	+
D-Mannose	+	+	+	+
L-Sorbose	-	-	-	-
L-Rhamnose	-	-	-	-
Dulcitol	-	-	-	-
Inositol	-	-	-	-
D-Mannitol	-	-	+	+
D-Sorbitol	-	-	-	-
Methyl-a-D-mannopyranoside	-	-	-	-
Methyl-a-D-glucopyranoside	+	+	+	+
N-acetylglucosamine	+	+	+	+
Amygdalin	+	+	+	+
Arbutin	-	-	+	+
Esculin	+	+	+	+
Salicin	+	+	+	+
D-cellobiose	+	+	-	+
D-maltose	+	+	+	+
D-lactose	-	-	+	+
D-melibiose	+	+	+	+
Sucrose	+	+	+	+
D-Trehalose	+	+	+	+
Inulin	-	-	-	-
D-Melezitose	-	-	-	-
D-Raffinose	+	+	+	+
Amylose	-	-	-	-
Glycogen	-	-	-	-
Xylitol	-	-	-	-
Gentiobiose	-	-	-	-
D-Turanose	+	+	+	+
D-Lixose	-	-	-	-
D-Tagatose	-	-	-	-
D-Fucose	-	-	-	-
L-Fucose	-	-	-	-
D-Arabitol	-	-	-	-
L-Arabitol	-	-	-	-
Potassium Gluconate	-	-	+	-
Potassium 2-ketogluconate	-	-	+	-
Potassium 5-ketogluconate	-	-	+	-

RCTw1.1 and DRC1506: *Ln. mesenteroides* ssp. *jonggajibkimchii.* DSM20241: *Ln. mesenteroides* ssp. *suionicum*

Tw234 and RCTw1.1 strains displayed the same fermentation profile. Unlike the collection strains DRC1506 and DSM20241, both strains did not use xylose, mannitol, arbutin, and lactose as carbon sources. Tw234 and RCTw1.1 strains were able to ferment D-cellobiose, as described for the DSM20241 strain but not for the DRC1506 strain.

A remarkable data derived from the same information source indicates that the strain type DRC1506 can ferment gluconate, 2-ketogluconate, and 5-ketogluconate as potassium salts. This physiological trait is not described in any of the subspecies of *Ln. mesenteroides* (Chun *et al*., 2017). The Tw234 and RCTw1.1 strains, as well as the *Ln.mesenteroides* ssp. strain DMS20241 were not able to use the sugars as mentioned earlier as energy sources.

Phylogenetic tree analysis based on 16S rRNA gene sequences shows that strains *Ln. mesenteroides* ssp. *jonggajibkimchii* DRC1506, Tw234 and RCTw1.1 are grouped in the same clade and very close to the other subspecies of *Ln. mesenteroides* (Figure 1). 16S rRNA of the Tw234 strain (1233 bp) exhibited 100% homology with the sequence of the strain type *Ln. mesenteroides* ssp. *jonggajibkimchii* DRC1506, using the BLAST program (Basic Local Alignment Search Tool). The cited type strain can be found in the Japanese Collection of Microorganisms under the JCM 31787 denomination or in the Korean Culture Center of Microorganisms under the KCCM 43249 denomination. The partial sequence of the gene 16S rRNA of the strain under study was deposited in the GenBank under the name *Ln. mesenteroides* ssp*. jonggajibkimchii* Tw234 (access number: MN831890.1).

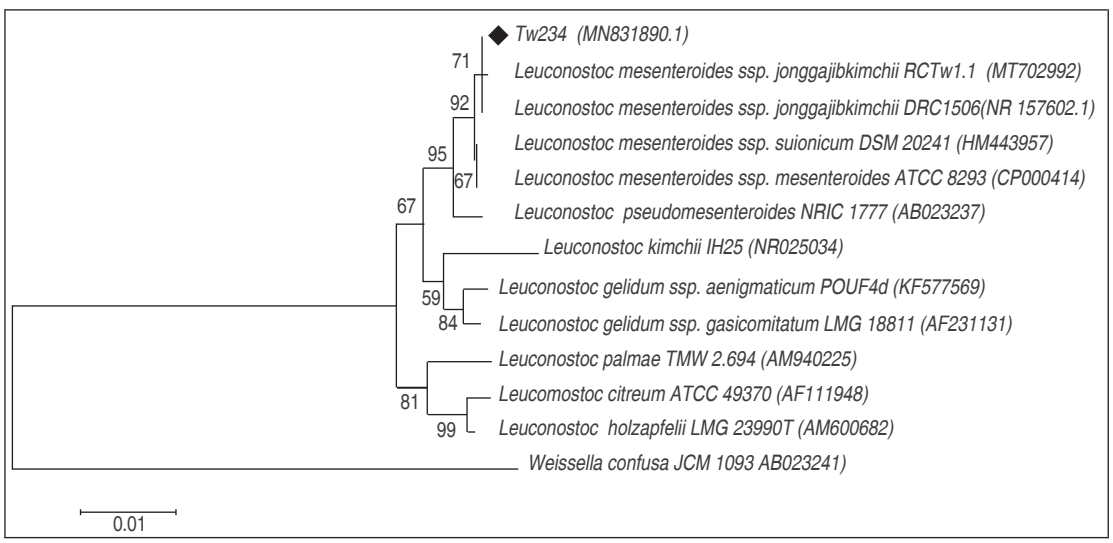

Figure 1. Phylogenetic tree constructed by Neighbour-Joining method based on the relationship between the 16S rRNA gene sequences of strain Tw234 (♦) and related species with the genus *Leuconostoc*. The numbers at internal nodes are bootstrap support values ($\geq$70%). GenBank accession numbers are given in parentheses. The 16S rRNA sequence of *Weissella confusa* was chosen arbitrarily as the outgroup sequence (bar, 0.01 substitution per nucleotide position).

Figure 2a displays the change in pH values in Chinese cabbage and white cabbage fermentation when the process was carried out by the Tw234 strain. The values remained relatively stable for the first 12 h of the process and then dropped until stabilized at 48 h. Chinese cabbage and white cabbage reached pH values of 4.27 and 3.89, respectively, remaining stable until 96 h.

Figure 2b shows the changes that occurred when the process was performed with the RCTw1.1 strain. The pH decline began at 6 h and stabilized at 48 h, reaching values of 4.23 in Chinese cabbage and 3.69 in white cabbage. The maximum acidification rates of the Tw234 strain were 0.047 and 0.074 pH h^{-1} units for Chinese and white cabbage, respectively, while values of 0.037 and 0.068 pH h^{-1} units were determined for the RCTw1.1 strain.

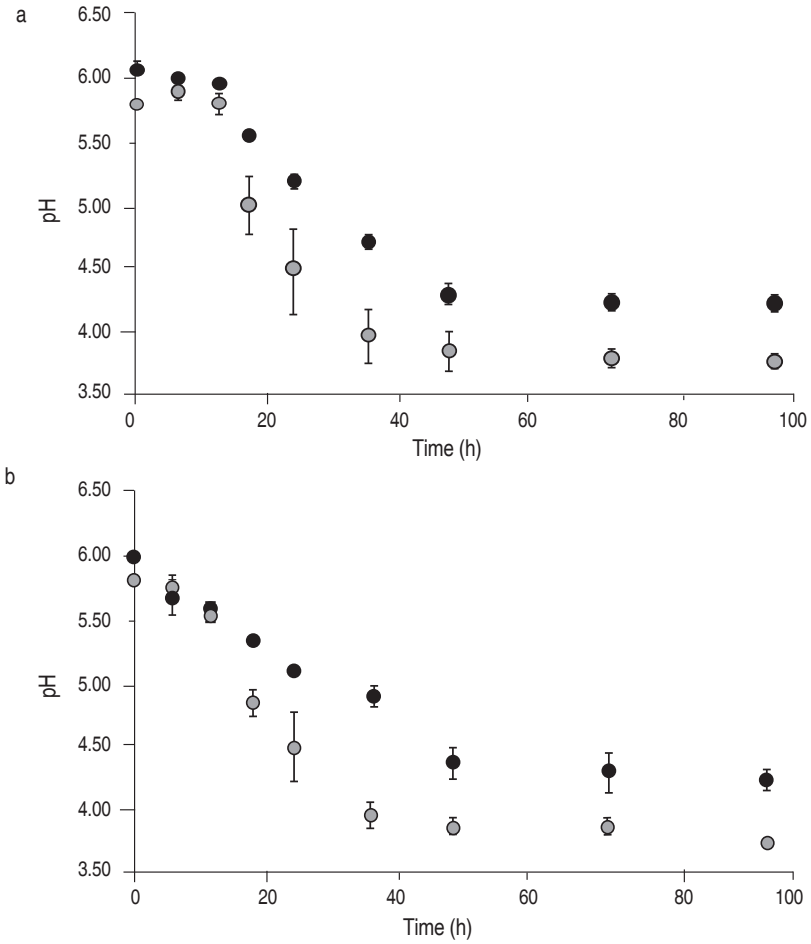

Figure 2. Changes in pH values during fermentation of white cabbage (◦) and Chinese cabbage (•) inoculated with the strains Tw234 (a) and *Ln. mesenteroides* ssp. *jonggajibkimchii* RCTw1.1 (b). Each value is the mean ± standard deviation of two measurements.

The final pH values and maximum acidification rates are comparable in both strains. The differences observed when comparing the evolution of the two vegetables' parameters are due to the different supply of sugars that are at a much higher concentration in white cabbage compared to Chinese cabbage (USDA, 2020).

The final pH and acidification rate values obtained in the fermentation of white cabbage using the Tw234 strain, are comparable to those reported by Johanningsmeier *et al.* (2007) when the *Ln. mesenteroides* strain LA 81 (ATCC 8293) was used as a starter. Previous reports recommend, to obtain good quality kimchi, the use of strains that adapt to the environment generated during the fermentation of Chinese cabbage (low temperatures, low pH, and presence of NaCl) and also, that the final pH does not drop below 4.2 (Ick, 2003; Jung *et al.*, 2014). The Tw234 strain exhibits all these characteristics, may be of interest to the food industry, its potential use as a starter in producing foods that include the cited cruciferous.

Several microbial growth models are found in the literature, such as the Baranyi, Logistic, and Gompertz models (Zwietering *et al.*, 1990). In this study was used Gompertz model, regarded as the most suitable model to describe microbial growth curves due to its simplicity and interdependence of the parameters. Growth curves of the strains *Ln mesenteroides* ssp. *jonggajibkimchii* Tw234 and RCTw1.1 were satisfactorily modeled using the Gompertz equation (1) cited above, obtaining R^2_{adj} values between 0.98 and 0.99, and low RMSE values (0.13–0.36) (Table 2).

In Figure 3, experimental data and growth curves modeled for both strains can be observed during the fermentation

Table 2. Value of parameters obtained by non-linear regression of Gompertz equation (1) for the growth of the strains *Ln. mesenteroides* ssp. *jonggajibkimchii* Tw234 and RCTw1.1 under study.

Parameters	Chinese cabbage		White cabbage	
	Tw234	RCTw1.1	Tw234	RCTw1.1
A	7.15±0.24 a	5.94±0.25 b	8.07±0.04 a	7.22±0.77 a
μ_m	0.70±0.02 a	0.83±0.10 a	0.78±0.02 a	0.70±0.15 a
λ	10.41±1.28 a	11.53±0.72 a	10.76±0.28 a	10.10±2.19 a
R^2_{adj}	0.998	0.989	0.999	0.985
RMSE	0.13	0.29	0.13	0.36

A: log increase in population; μ_m: maximum growth rate (log CFU mL^{-1} h^{-1}); λ: lag phase (h).
Mean value ± standard deviation. Means followed by the same letter have not a statistically significant difference (P<0.05) by Tukey test

of Chinese cabbage and white cabbage. The estimated parameters μ_m (specific growth rate) and λ (latency phase duration) showed no statistically significant differences between the strains studied (P>0.05). The asymptotic value (Figure 3a) showed significant differences among cultures only in Chinese cabbage (P<0.05), getting the strain Tw234 ($\log_{10}$ 7.15±0.24) a higher increase in population than the strain RCTw1.1 ($\log_{10}$ 5.94±0.25).

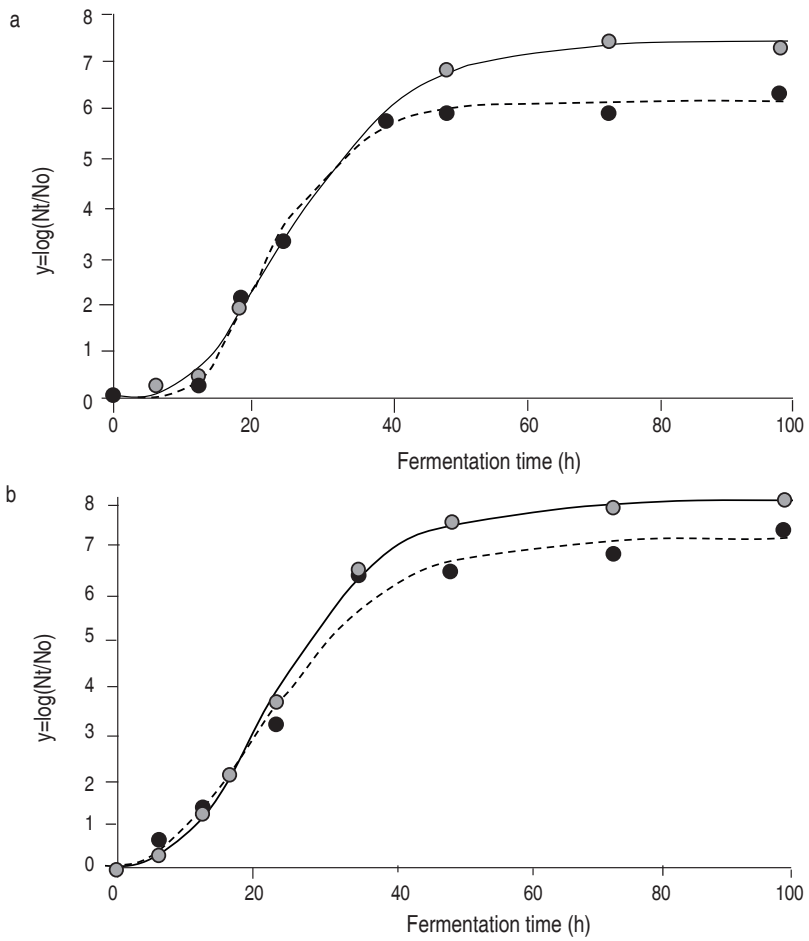

Figure 3. Time course for the growth of the strains Tw234 (∗) y *Ln. mesenteroides* ssp. *jonggajibkimchii* RCTw1.1 (•) in Chinese cabbage (a) y white cabbage (b). Dots represent experimental data, and lines show the bacterial growth curve modeling.

CONCLUSIONS

In recent years, the search and selection of LAB based on technological, sensory, and nutritional characteristics for application in controlled plant fermentations have been developed with intensity.

The goal of the selection of strains for the controlled production of fermented vegetables is to obtain a uniform quality product that avoids variations in spontaneous processes. The patent for *Ln. mesenteroides* ssp. strains *jonggajibkimchii* DRC1506 and *Ln. mesenteroides* ssp. *suionicum* DSM20241 marketed by Korean company Daesang for kimchi production is an example of this trend. In this work, the first isolation of a strain *Ln. mesenteroides* ssp. *jonggajibkimchii* from the intestinal content of Parona leatherjacket (*Parona signata*) were reported. The strain exhibits comparable characteristics with the cabbage isolated strain RCTw1.1 and the type of strain *Ln. mesenteroides* ssp. *jonggajibkimchii* DRC1506. This study also demonstrated that the strain Tw234 exhibits the metabolic characteristic of interest to the food industry as growing at low temperatures and exopolysaccharides production. Moreover, the evaluated kinetic parameters in vegetable matrices were similar between the RCTw1.1 and Tw234 strains, achieving this latter a better fit to the kinetic model. These properties make *Ln. mesenteroides* ssp. *jonggajibkimchii* Tw234 a potential candidate to be used as a starter in controlled fermentations. *Leuconostoc* and related species start the first stage of vegetable fermentation; therefore, only the metabolic traits related to this phenomenon were investigated in this work. However, new technological features such as synthesis of antimicrobial compounds, increase of the antioxidant activity, behavior at industrial scale, and combination with other strains in two-stage controlled fermentation should be approached in future investigation.

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Short article

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Assessing the influence of cereal-legume mixtures on the productivity of degraded pastures in the Kostanay region of northern Kazakhstan

Evaluación de la influencia de las mezclas de cereales y leguminosas en la productividad de pastos degradados en la región de Kostanay, en el norte de Kazajstán

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ABSTRACT

This study presents the results of some options to restore pastures with low productivity in the arid steppes of the Kostanay region of northern Kazakhstan, describing the effects associated with grass-legume mixtures. The effects of grass-legume mixtures, contribution to the preservation and maintenance of pasture forage crops, and the productivity of pastures were discussed. Mixtures of grasses and legumes were selected that are promising crops for arid regions.The plant density and its condition were determined based on test plots of adjacent rows of 0.5 m each, followed by counting. The plant height was determined before the yield of green mass by measuring 25 plants of each species. The yield of green mass in the maturity phase of the grass was determined by mowing and weighing the green mass in the plots, followed by the analysis of the species composition in the grass mixture and drying until air dry. The density of plants, the height of the plants, and the safety of the forage plants according to the sowing method were the data collected. In addition, the effect of grass mixtures on the productivity of forage crops to improve pastures was compared. According to these results, the highest productivity under experimental conditions was found in the wheat grass-alfalfa-bromegrass variant. This information can contribute to the improvement of the state of the pastures since it is complete and inexpensive food for farm animals.

Keywords:
Fodder production
Grass-legume mixtures
Grasses
Pastures
Perennial plants

RESUMEN

Este estudio presenta los resultados de algunas opciones para restaurar pastos con baja productividad en las tierras áridas de la región de Kostanay (norte de Kazajstán). Se discutieron los efectos de las mezclas de gramíneas y leguminosas, la contribución a la preservación y mantenimiento de los cultivos forrajeros de pastos y la productividad de los mismos. Se seleccionaron mezclas de gramíneas y leguminosas que son cultivos prometedores para las regiones áridas. El estado de la planta y su densidad se determinaron con base en parcelas de prueba de hileras adyacentes de 0.5 m cada una, seguidas de conteo. La altura de la planta se determinó antes del rendimiento de masa verde (masa fresca) midiendo 25 plantas de cada especie. El rendimiento de masa verde en la fase de madurez del pasto se determinó cortando y pesando la masa fresca en las parcelas, seguido del análisis de la composición de especies en la mezcla de pasto y secando las gavillas al aire. La densidad de plantas, la altura de las plantas y la inocuidad de las plantas forrajeras según el método de siembra fueron los datos recogidos. Además, se comparó el efecto de las mezclas de gramíneas sobre la productividad de cultivos forrajeros para mejorar los pastos. Según estos resultados, la mayor productividad en condiciones experimentales se encontró en la variante trigo-alfalfa-hierba-bromo.. Esta información puede contribuir al mejoramiento del estado de los pastos, ya que es alimento completo y económico para los animales de granja.

Palabras clave:
Producción forrajera
Mezclas de hierbas-leguminosas
Pastos
Gramíneas
Plantas perennes

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The current conditions of pastures in many countries is unsatisfactory due to a strong degradation of vegetation and soil observed worldwide including North and South America, Africa, and Australia (Baethgen *et al*., 2020; Fuglie *et al*., 2021). This is due to the misuse of pastures in farms since continuous and irregular grazing is performed, generating a decrease in vegetation cover and regrowth of weeds and non-food plants. The Republic of Kazakhstan is an agrarian country and ranks sixth in the world in terms of pasture area (Nugmanov *et al*., 2018a). The total land area of pastures is 187 million ha. Currently, due to long-term unsystematic use, 48.0 million ha are degraded, including 26.5 million ha that are destroyed (Nugmanov *et al*., 2018a).

For the development of animal husbandry in Kazakhstan, it is necessary to create sustainable forage-based food. Given its potential, there are opportunities to introduce highly productive fodder crops and efficient technologies into agricultural production (Baranowski *et al.,* 2020). In Kazakhstan, the sources of plant feed for farm animals are pastures (187 million ha), hayfields (5.0 million ha), and fodder arable land (2.5 million ha) (Popov *et al*., 2017). Within the pasture maintenance of livestock, the rational maintenance of pastures is highly relevant, since the productivity of pasture lands with their correct use increases greatly (Popov *et al*., 2017).

After grazing, fodder cereals and forbs are removed from the grass stand while low-value, poisonous, and weed plants are introduced (Jun Li *et al*., 2007; Vasques *et al*., 2019). Researchers have established criteria and indicators of the degree of degradation of vegetation cover (Ministério da Agricultura e Reforma Agrária Brasil, 2009; Derpsch *et al*., 2010; Asai *et al*., 2018; Sereia *et al*., 2016; Viaud *et al*., 2018; Van Dyke *et al*., 2004; Soares *et al*., 2019; Dymova, 2006; Sizykh, 2007; Bazha *et al*., 2008). They include the low degree, which shows signs of degradation in dry years; moderate degree, which is a decrease in the yield, displacement of rare plants, and the appearance of weeds; high degree, which includes a decrease in productivity, seasonality of use, and displacement of the main types of plants. Very high degree indicators include severe shortages and infestation with undesirable species. The limiting influence of abiotic factors on the productivity of degraded pastures, is considered, as well as the influence of grazing as the most important deflationary process.

In the research of the Humanities and Economics Academy (HEA) member G. V. Blagoveshchensky, the use of a mixture of grass on pastures provided some advantages, such as reducing the incidence of diseases and pests, enriching the soil with organic substances, and leading to a sustainable harvest (Blagoveshchenskiy, 2013; Serekpayev *et al*., 2018). Restoration of degraded pastures in Brazil can improve livestock production and help avoid deforestation and should be a priority strategy for the agribusiness sector (Feltran-Barbieri and Feres, 2021).

The most relevant theoretical and methodological aspects of the biological characteristics of legumes and cereals, their cultivation, adaptation to soil and climatic conditions, and the impact on the productivity and quality of crops have been addressed. Sufficient attention was paid to the peculiarities and problems of the cultivation of perennial grasses, but the issue of the selection of legumes in the composition of pasture mixtures for the arid conditions of the Western Ciscaucasia (Bedilo, 2016).

The formation of grasses and legumes in the pasture due to the replacement of technical nitrogen with a biological source contributes to a decrease in the average annual anthropogenic costs by 40%. The main problems of the feed industry are low yields of pasture forage and a low level of use of pastures and hayfields (Tokusheva and Nugmanov, 2016; Tokusheva *et al*., 2017).

The relevance of the present study is associated with the achievement of high pastures productivity and long-term preservation of grasslands and the supply of food for animals. The main aim of this research was to study the effect of mixtures of grasses and legumes with different sowing methods on the productivity of degraded pastures in the arid steppe of northern Kazakhstan.

MATERIALS AND METHODS

Climatic and soil characteristics

The research location was Zarechnoye Agricultural Experimental Station LLP (AES LLP), which has been a

scientific organization and an elite seed farm since 1962. The main activity of the organization has been scientific support of the main activity of the agricultural formations in plant growing and animal husbandry; production of elite seeds of crops, which cover up to 40% of agricultural needs of the local farms in seeds, corresponding to the first class of the sowing standard. The enterprise has introduced more than 40 varieties of oilseeds and potatoes. It stores the gene pool of potatoes, oil flax, spring rape, sunflower, soybeans. Zarechnoye AES LLP closely cooperates with Kazakh and foreign scientific institutions and agricultural producers, also providing training and consulting services to agribusiness entities.

Zarechnoye AES LLP is located in Northern Kazakhstan, between the latitude 53°14'06"N and longitude 63°44'02"E. The region occupies a vast territory, about 114,000 km^2, which is subdivided into three natural and climatic zones (moderately arid steppe and forest-steppe, arid steppe, moderately dry steppe). The research institute is located in the second soil-climatic zone. This zone is represented by an arid steppe mainly with low-humus southern chernozems (Nugmanov *et al.,* 2018b).

The climate in the research area is sharply continental with hot and dry summers and cold winters with little snow. The annual amplitude of air temperature is on average 75 °C, and in some years, it has reached 88 °C. In winter, the minimum air temperature often drops to 35-40 °C, and in rare cases, the temperature drops to 45-50 °C. In summer, the absolute temperature rises to +41-43 °C. The warm period with an average daily temperature above 0 °C lasts 195-200 days (from April to October). The duration of the frost-free period ranges from 108 to 130 days. The average annual air temperature is 0.3-2.3 °C, and in some years, it rises to 4.5-5.0 °C or drops to 0-1.2 °C. The duration of the growing season increases from north to south and is 166 to 174 days. A characteristic feature of the continental climate is the predominance of precipitation during the warm period (May to October) when 60-80% of the annual rate falls. The maximum precipitation occurs in the second half of summer, most often in July. The moisture index (HTC) in the region varies from 0.9 in the north to 0.5 in the south. Prolonged cold in spring, an earlier cold snap in autumn, and rainfall in late summer are typical of the region's climate and distinguish it from other arid regions. High insolation, a sharp temperature difference between day and night, low air humidity, low cloudiness, and frequent winds cause intensive evaporation of moisture 2 to 5 times higher than the amount of precipitation.

The meteorological conditions in 2016 showed favorable conditions for the growth and development of perennial crops. The annual amount of precipitation in 2016 exceeded the average annual normal and amounted to 559.9 mm. In 2017, the annual precipitation exceeded the average annual precipitation rate and amounted to 425.9 mm, which favorably influenced the growth and development of perennial grasses. In 2018, the annual precipitation rate slightly exceeded the average annual precipitation rate and reached 382.2 mm, which also positively influenced the formation of the yield of pasture crops (Table 1).

Table 1. Distribution of precipitation by periods of the year in comparison with the long-term normal (2016-2018).

Year	Amount of precipitation (mm)			
	Total for the year (October to September)	Cold period (November to March)	Warm period (April to October)	During the growing season (May to August)
Long-term normal	340.0	98.0	242.0	162.0
2016	559.9	183.6	338.3	205.9
2017	425.9	123.5	285.7	234.4
2018	382.2	116.6	324.5	239.2

Snow precipitation represents 30-40% of the total annual amount, but the maximum precipitation falls in July and amounts to 55-70 mm. There are years when precipitation during the growing season is practically

absent, and in some years, it is 2-3 times higher than usual.

However, even a low temperature, compared to the long-term average norm, and an almost complete absence of precipitation, caused intensive evaporation of moisture from plants and the soil surface, which eventually influenced a reduction in the period of plant development phases and a decrease in their growing season (Table 2).

Table 2. Average monthly air temperature during the growing season for the 2016-2018 period, Kostanay region.

Months	Air temperature (°C)			
	2016	2017	2018	Long-term norms
May	13.8	13.5	11.9	13.7
June	18.3	18.7	16.6	20.0
July	20.3	19.7	22.1	20.9
August	22.9	20.3	18.1	18.9

When analyzing the air temperature in July, no strong deviations from the long-term norm were observed (-0.6, -1.2 and +1.2 °C), which can also be said about August (+4, +1.4 and -0.8 °C from the average annual rate). The soil of the experimental site is southern thin chernozem in a complex with solonetz up to 10%. The thickness of the humus horizon (A+B) equals 41 to 45 cm. Effervescence from HCl from 85 cm, release of carbonates from the same depth. The humus content is 3.0-3.2%. Soil sampling was carried out using a drill. Soil samples taken for laboratory analysis were prepared. Each sample was placed on paper, scattered in a thin layer, and dried. After that, the samples were sent to a chemical laboratory. According to the analyses carried out by the agrochemical laboratory of the Institute, the soil of the experimental plot contains 0.15-0.16% of total nitrogen (in the 0-20 cm layer) and 0.10-0.13% of phosphorus.

Mobile forms of nitrogen (NO_3) were measured in the soil using the Grandval-Lyazh method (22.5-25.5 mg kg^{-1} soil – average). This method is based on the interaction of nitrates with disulfophenolic acid with the formation of trinitrophenol (picric acid). In an alkaline environment, it gives a color yellow due to the formation of potassium trinitrophenolate (or sodium, depending on the alkali used) in the amount equivalent to the content of nitrates (23). Mobile compounds of phosphorus and potassium were determined using the Chirikov method. This method is based on the extraction of P_2O_5 and K_2O from the soil with a solution of acetic acid at a concentration of 1:2.5 (soil to solution) and the subsequent determination of phosphorus in the form of a blue phosphorus-molybdenum complex on a photoelectric colorimeter and potassium on a flame photometer (24). The phosphorus content was 114-136 mg kg^{-1} of soil (high) and potassium content – more than 200 mg kg^{-1} of soil (high). There is a small amount of exchangeable sodium and potassium. The reaction of the aqueous suspension within the first meter is slightly alkaline.

Research methods

Research and observations were carried out following generally accepted methods and state standards (GOSTs). These methods were the methodology of field experiment (Dospekhov, 1985) and methodological guidelines for conducting field experiments with forage crops (All-Russian Williams Fodder Research Institute) (Podgot *et al*., 1983). According to these methods, standing density accounting was performed twice: after germination, before harvesting on designated areas in the 1st and 3rd replicates. Plant density and safety were determined on test plots of adjacent rows of 0.5 m with subsequent counting. According to the technique for studying the grass height, measurements were made at five points on each replica, located at a distance of 1 m from each other (20 measurements). A rail with a centimeter scale was placed vertically among the plants, a bunch of plants was attached to it, and the height of the highest part of the plants was measured. The height

of the plants was determined before accounting for the green mass yield by measuring 25 plants of each species.

The botanical composition of the herbage was determined by analyzing plant samples weighing 1 kg with the isolation of legumes, cereals, and herbs, followed by weighing each component. To determine the botanical composition of the herbage, 100-250 g of air-dried mass or 500-1000 g of freshly harvested green forage mass were used. In a species analysis, each sample was disassembled into separate types of herbs. The fractions were weighed and the botanical composition of the herbage was determined as a percentage of the sample weight. The botanical composition of the herbage was determined before each grazing.

The yield of green mass in the maturity phase of pasture was determined by mowing and weighing the green mass in the log plots with an analysis of the species composition of the grass mixture and drying the sheaves until air dry. Experiments were performed in the field (Table 3). Plot

Table 3. Experiment setup.

		Sowing methods	
1	Degraded pasture land (control)	-	-
2	Wheatgrass (*Agropyron pectiniforme* Roem.et Schult.) — alfalfa (*Medicago sativa* L.) — bromegrass *(Bromus inermis* Leyss.*)*	Wide-row sowing — 27 cm	Ordinary sowing — 15 cm
3	Wild rye (*Elymus junceus* Fisch.) — alfalfa (*Medicago sativa* L.) -bromegrass (*Bromus inermis* Leyss.)		
4	Slender wheatgrass (*Elymus trachycaulus* Get.S.) — alfalfa (*Medicago sativa* L.) — wheatgrass (*Agropyron pectiniforme* Roem.et Schult.).		

area was set by SKP-2.7 seeder (anchor opener) — 97.2 m^2 and Wintersteiger machine (disc opener) — 60.0 m^2. The experiment was repeated 4 times.

In the spring, on the degraded site where the experiments were laid, a survey of the vegetation cover and the soil cover was carried out. In the summer, after the vegetation regrowth, a repeated survey was carried out, and the species composition of the vegetation was determined. After that, the rate of consumption of the continuous herbicide was calculated. A continuous herbicide was applied for 8-10 days before sowing perennial grasses. Then, as soon as the vegetative mass dried up and the weeds died, the BZTs-12 tooth harrow machine was used at the site of the experiments without disturbing the soil sod. In the presence of moisture at a sowing depth of 2-3 cm, perennial grasses were sown directly onto the lawn with SKP-2.7 seeders (anchor opener) using the wide-row sowing method and Wintersteiger (disc opener) using the ordinary row sowing method. The varietal composition of the perennial grasses when the experiments were performed was Batyr wheatgrass, Akmolinsky 91 bromegrass, Shortandinsky wild rye, Arman slender wheatgrass, Raikhan alfalfa.

Static data processing was performed by analysis of variance using Microsoft Excel and AGROS 2.11. This method is used to assess the significance of differences between several groups of observations.

RESULTS AND DISCUSSION

The density of plants is influenced by factors such as weather conditions, soil fertility, biological characteristics of plants, seeding rate. According to Tiscornia *et al.* (2019), more arid pastures, as a rule, depend on the climatic conditions. The development and observation phases are carried out depending on the crops. The plant density of grass-legume mixtures during the evaluating period with wide-row and ordinary sowing methods is shown in Table 4. In the course of the

standing plants density studies, in the degraded pasture control plot, herbs such as fescue, feather grass, and cold wormwood were noticed. In addition, in each variant of the experiment, natural vegetation was found, which was not counted in the calculation of the number of plants.

Table 4. The standing density of grass-legume mixtures of the first year of life (2016-2018).

Experiment variants	Number of plants (pieces m^{-2})											
	Wide-row sowing method						Ordinary row sowing method					
	2016		2017		2018		2016		2017		2018	
	by crop	total	by crop	total	by crop	total	by crop	total	by crop	total	by crop	total
Degraded pasture land (control)	50		56		53		50		56		53	
Wheatgrass-alfalfa-bromegrass	47 36 42	125	46 34 40	120	44 32 37	113	98 77 95	262	94 72 92	258	90 65 86	241
Wild rye-alfalfa-bromegrass	45 31 43	119	43 28 41	112	40 26 39	105	95 75 92	259	92 73 90	255	89 68 84	241
Slender wheatgrass-alfalfa-wheatgrass	46 28 45	119	45 26 44	115	43 24 42	109	94 68 97	270	92 64 95	251	89 58 91	238

In 2017, the density on the wheatgrass-alfalfa-bromegrass variant equaled 46, 34, and 40 pieces m^{-2} and in grass mixtures 120 pieces m^{-2}. These values are similar to the previous year, which suggests that the meteorological conditions did not differ much. In 2018, the highest value was also noted for the wheatgrass-alfalfa-bromegrass variant (44, 32, and 37 pieces m^{-2}), while the grass mixtures were 113 pieces m^{-2}.

The conservation of perennial grasses after the winter period in mixed legumes and cereals crops differed in the variants with wide-row and ordinary row sowing methods. The preservation of perennial forage grasses depends primarily on how the crop wintered. As shown in Table 5, data on the safety of perennial grasses for 2016-2018 are given with ordinary row sowing methods, where the results showed significantly good values in comparison with the wide row sowing method. The greatest indicator of the safety of grass-legume plants was noted in the wheatgrass-alfalfa-bromegrass variant (91%) and the wild rye-alfalfa-bromegrass variant (90%). The highest preservation rate of grass-legume mixtures of the first year of life on average for 3 years (2016-2018) was observed for wheatgrass-alfalfa-bromegrass with 85% for the wide-row sowing method and 91% for the ordinary row sowing method. With this method of sowing, the plots showed good preservation, since the plants were located at a distance of 15 cm and this had the advantage of preserving the plants from the winter cold.

The height of plants is influenced by agrometeorological conditions, soil fertility, and agricultural cultivation techniques. To measure the height of plants, the start and end dates of the measurement must be observed. For annual perennial sown grasses, as well as grass mixtures, it was necessary to perform measurements at the beginning of the term when a height of 5 cm was reached in spring, and after cutting when young shoots 5 cm long appear.

Table 5. Preservation of grass-legume mixtures after the winter period (2016-2018)

Experiment variants	With a wide-row sowing method							With an ordinary row sowing method						
	2016		2017		2018		Preservation (2016-2018)	2016		2017		2018		Preservation (2016-2018)
	%													
Degraded pasture land (control)	25		28		27		27	25		28		27		27
Wheatgrass-alfalfa-bromegrass	94 90 84	89	92 85 80	86	88 80 74	81	85	98 96 95	96	94 90 92	92	90 81 86	86	91
Wild rye-alfalfa-bromegrass	90 77 86	84	86 70 84	80	80 65 78	74	80	95 94 92	94	92 91 90	91	89 85 84	86	90
Slender wheatgrass-alfalfa-wheatgrass	92 70 90	84	90 65 88	81	86 60 84	77	81	94 85 97	92	92 80 95	90	89 73 91	85	89

The last measurement of the height was necessary to carry out the beginning of haymaking in the study area or at the beginning of grazing (cessation of growth).

The height of grass-legume mixtures in 2016 varied from 7 to 91 cm depending on the sowing method, as shown in Table 6. On the control plot of degraded pasture land, the plant height was 9 cm in 2016, 10 cm in 2017, 12 cm in 2018, on the variants of grass mixtures with a wide-row sowing method the wheatgrass-alfalfa-bromegrass variant had the following plant height parameters during 2016: 11 cm for cereals, 8 cm for legumes, and 9 cm for natural vegetation. On the experimental site, natural vegetation was found, namely fescue, feather grass, and cold glade. For the wild rye-alfalfa-bromegrass variant, the height of cereal plants was 12 cm, 14 cm for legumes, and 10 cm for natural vegetation; for the slender wheatgrass-sainfoin-wheatgrass variant, the height of cereal plants equaled 11 cm, 13 cm for legumes, and 10 cm for natural vegetation. The plant height was also considered for the plot with the ordinary sowing method in 2016 (Table 6).

Table 6. Height of grass-legume mixtures of the first years of life, depending on the method of sowing.

Experiment variants	Plant height (cm)					
	Wide-row sowing method			Ordinary row sowing method		
	2016	2017	2018	2016	2017	2018
Degraded pasture land (control)	9	10	12	9	10	12
Wheatgrass-alfalfa-bromegrass	C-11 L-8 N-9	C-15 L-10 N-9	C-13 L-11 N-10	C-8 L-7 N-10	C-16 L-9 N-10	C-15 L-12 N-11
Wild rye-alfalfa-bromegrass	C-12 L-14 N-10	C-13 L-11 N-10	C-12 L-13 N-10	C-9 L-7 N-13	C-17 L-12 N-11	C-16 L-14 N-11
Slender wheatgrass-alfalfa-wheatgrass	C-11 L-13 N-10	C-12 L-9 N-11	C-10 L-11 N-9	C-10 L-11 N-11	C-16 L-10 N-12	C-13 L-10 N-8

C: cereals, L: legumes, N: natural vegetation (fescue, feather grass, cold wormwood).

With the ordinary row sowing method, there was a positive growth of the grass-and-legume mixture in height. In 2018, the growth and development indicators of grass-legume mixtures were moderate, since forage crops provide a good vegetative mass in subsequent years of life.

To determine the yield of grass-legume mixtures, a selection was carried out according to the medium sheaf samples selection method (1 kg of each plot). The yield of the evaluating period of life is shown in Table 7 with both methods, The productivity of perennial grasses of moderately winter-hardy grass-legume mixtures with different sowing methods in 2016 had insignificant differences since the amount of precipitation during the growing season was 205.9 mm, which is several times higher than the long-term norm. The favorable air temperature contributed to this result. With an ordinary method of sowing in 2016, the wheatgrass-alfalfa-bromegrass obtained a yield of 440 kg ha^{-1}, and the yield of slender wheatgrass-alfalfa-bromegrass was 420 kg ha^{-1}.

Table 7. The average productivity of grass-legume mixtures during 2016-2018.

Experiment variants	kg ha^{-1}							
	wide-row sowing method				ordinary row sowing method			
	2016	2017	2018	Average (2016-2018)	2016	2017	2018	Average (2016-2018)
Degraded pastureland (control)	100	150	180	140	100	150	180	140
Wheatgrass-alfalfa-bromegrass	480	500	470	480	440	480	460	460
Wild rye-alfalfa-bromegrass	480	490	450	470	420	460	440	440
Slender wheatgrass-alfalfa-wheatgrass	440	460	420	440	400	440	400	410
Least significant difference$_{05}$				14				12

These results show that concerning the productivity of the air-dry mass of perennial grasses, the highest yield in 2017 was obtained on variants such as wheatgrass-alfalfa-bromegrass (500 kg ha^{-1}), wild rye-alfalfa-bromegrass (490 kg ha^{-1}), and slender wheatgrass-alfalfa-wheatgrass (460 kg ha^{-1}). The yield of perennial crops was slightly different. Comparing the variants of the experiment with the control of degraded pastureland, it can be seen that the variants are twice superior to the rest. With ordinary sowing methods in 2017, the productivity of the air-dry mass in the variant wheatgrass-alfalfa-bromegrass with 480 kg ha^{-1}, wild rye-alfalfa-bromegrass amounted was 460 kg ha^{-1} and 440 kg ha^{-1} for slender wheatgrass-alfalfa-wheatgrass. Although that year, according to meteorological data, it did not differ much from the long-term norms. Nevertheless, it can be assumed that the productivity of grass mixtures depends not only on climatic conditions but also on crop safety.

According to the data obtained, the yield of grass-and-legume mixtures with a wide-row sowing method in 2018 had the following values for the following variants: wheatgrass-alfalfa-bromegrass — 470 kg ha^{-1}, wild rye-alfalfa-bromegrass — 450 kg ha^{-1}, slender wheatgrass-alfalfa-wheatgrass — 420 kg ha^{-1}, compared with the control plot.

On average for 2016-2018 the highest yield was observed on the wheatgrass-alfalfa-bromegrass variant (480 kg ha^{-1}). With an ordinary row sowing method, the productivity of grass-legume mixtures on average for 2016-2018 showed an insignificant difference, which was observed on the wheatgrass-alfalfa-bromegrass variant (460 kg ha^{-1}). The wide-row sowing method is used for plants that require a large area of nutrition and are heavily clogged with weeds and grow slowly after sowing. Ordinary row sowing methods are the most ideal ones since the seeds are embedded in the soil

at the same time. The advantage of these methods is that the seeds are placed in optimal and uniform conditions, which ensures uniform germination and uniform emergence of seedlings. This is used in the development of a resource-saving technology for the surface improvement of pastures, which is achieved by selecting crops of perennial cereals and legumes of forage grasses, as well as direct sowing of their mixtures into the sod, which allows to reduce energy costs and provides in the steppe zone the receipt of up to 1500 kg ha^{-1} of pasture mass (Serekpaev *et al*., 2016). Havilah, (2011) found that pasture and annual forage grasses can provide animals with high-yielding and high-quality forage. Australian scientists (Fulkerson *et al*., 2011) stated that alfalfa mixtures create and maintain pastures for long-term use. In a Mediterranean setting (Bathgate *et al.,* 2009), new annual legume grasses can affect farm profits and land use and result in a 26% increase in farm profits. Thus, cereal-legume grass mixtures can increase the productivity of degraded pastures.

CONCLUSIONS

As a result of the research, it can be concluded that the productivity of grass-legume mixtures of the first years of life with wide-row and ordinary row sowing methods, the highest result was noted on the average (2016-2018) for the wheatgrass-alfalfa-bromegrass variant (480 kg ha^{-1}, 460 kg ha^{-1}). The use of resource-saving methods allows preventing the degradation of pasture herbage and will also help to improve the quality of forage, yield, and soil fertility. In comparison with the control, the experimental variants showed high productivity. Future scope of research could include monitoring of the study area, assessing the safety of cereal-legume grass mixtures, and improving degraded pastures.

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ÍNDICE DE AUTORES

POLÍTICA EDITORIAL

REVISTA FACULTAD NACIONAL DE AGRONOMÍA MEDELLÍN

La Revista Facultad Nacional de Agronomía Medellín (RFNA), es una publicación de la Facultad de Ciencias Agrarias de la Universidad Nacional de Colombia - Sede Medellín. Esta orientada a profesores, investigadores, estudiantes, extensionistas y a todos aquellos profesionales que crean conocimiento y articulan la ciencia y la tecnología para hacer más productivo el campo a nivel empresarial y de economía campesina.

La Revista recibe y publica, sin ningún costo, artículos en idioma inglés de investigación, revisión, reseñas, cartas al editor y editoriales.

La periodicidad de la Revista es cuatrimestral, con circulación nacional e internacional y tiene como objetivo divulgar artículos escritos en inglés, originales, inéditos y arbitrados (peer review) de carácter científico que respondan a preguntas específicas y que proporcionen soporte y pruebas a una hipótesis, en aspectos relacionados con las Ciencias Agronómicas, Zootecnia, Ciencias Forestales e Ingeniería Agrícola y de Alimentos y otras afines que contribuyan a la solución de los limitantes del agro en el trópico.

Teniendo en cuenta los criterios considerados por Colciencias, la revista acoge documentos de las siguientes tipologías:

Artículos de investigación científica y tecnológica: Documento que presenta, de manera detallada, los resultados originales de proyectos terminados de investigación. La estructura generalmente utilizada contiene cuatro partes fundamentales: Introducción, metodología (materiales y métodos), resultados y discusión, y conclusiones. La extensión máxima debe ser de 5200 palabras, excluyendo figuras, tablas, referencias. El mínimo de referencias bibliográficas sugerido es de 10 y el máximo de 30. Este tipo de artículos es arbitrado e indexado.

Artículos de revisión: Documentos producto de una investigación terminada donde se analizan, sistematizan e integran los resultados de investigaciones publicadas o no publicadas, sobre un campo en ciencia o tecnología, con el fin de dar cuenta de los avances y las tendencias de desarrollo. Se caracteriza por presentar una cuidadosa revisión bibliográfica de por lo menos 50 referencias. La extensión máxima debe ser de 6000 palabras, excluyendo figuras, tablas, referencias. Este tipo de artículos es arbitrado e indexado.

Artículos cortos: Documento breve que presenta resultados originales preliminares o parciales de una investigación científica o tecnológica, que por lo general requieren de una pronta difusión. Para todos los casos el 60% de las citas debe provenir de artículos publicados en los últimos diez años.

Los artículos deben ser presentados de acuerdo a los lineamientos establecidos en las “Instrucciones a los Autores”; quienes incumplan las normas básicas no iniciarán el proceso editorial. Se debe diligenciar el formato “Autorización para Publicación de Obras y Cesión de Derechos Patrimoniales”, el cual será suministrado por la Revista. Dicho documento es explícito en mencionar que todos los autores están informados y de acuerdo con someter el artículo a consideración de la Revista, que no hay conflictos de interés entre ellos y expresa que el contenido del manuscrito no ha sido ni será enviado para su publicación a otra Revista.

El Comité Editorial, apoyado por un equipo de editores asociados, evaluará el mérito científico del documento y luego lo someterá a evaluación bajo la modalidad doble ciego -es decir que se guarda estricto anonimato en la revisión- por dos árbitros especializados en el tema, preferiblemente uno nacional y otro internacional, quienes entregarán su dictamen en el formato establecido por la Revista. El Comité Editorial se reserva el derecho de aceptar o no las colaboraciones. El dictamen luego del proceso de revisión puede ser: aceptado para publicación sin ninguna o pocas modificaciones; aceptado para publicación con cambios mayores de acuerdo a las observaciones de los evaluadores; reconsiderado para publicación si se modifica sustancialmente, en este caso, será catalogado como material nuevo; rechazado para publicación. Si los artículos son aceptados, estos serán devueltos a los autores para su corrección y remitidos de nuevo al Director de la Revista en los siguientes 30 días calendario.

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INSTRUCCIONES A LOS AUTORES

Lineamientos generales

Los artículos deben ser enviados a través del Open Journal System en el Portal de Revistas de la Universidad Nacional de Colombia http://www revistas.unal.edu.co/. Sólo serán considerados artículos escritos en inglés. Adjunto se deben remitir los siguientes cuatro formatos: (1) Lista de verificación de criterios editoriales para la presentación de manuscritos; (2) Autorización de publicación de manuscritos en la Revista Facultad Nacional de Agronomía Medellín, en la cual se acepta la no postulación simultánea del artículo a otras revistas u órganos editoriales y se ceden los derechos a la Revista para su difusión, este debe ser firmado por todos los autores del manuscrito; (3) Datos personales de cada autor; (4) Sugerencia de posibles pares evaluadores. Las formas de publicación son: artículos de investigación científica y tecnológica, artículos de revisión y artículos cortos. Los artículos pueden ser elaborados por profesores y/o investigadores de la Universidad Nacional de Colombia, o cualquier otra institución afín, nacional o internacional, en los temas Agropecuarios, Forestales y de Ingeniería Agrícola y de Alimentos. El manuscrito no debe exceder 5200 palabras para artículos de investigación y 6000 para artículos de revisión. Las hojas deben ser tamaño carta, escritas a interlineado doble, numeración de línea continua, letra o fuente Times New Roman o Verdana, tamaño 12 puntos, márgenes de 3 cm en la parte superior, 2 cm en la inferior y 2,5 cm en las márgenes laterales derecha e izquierda. Las tablas y figuras (es decir, los gráficos, dibujos, esquemas, diagramas de flujo, fotografías y mapas) se deben mostrar incorporadas en el texto y con numeración consecutiva (Tabla 1... Tabla n; Figura 1... Figura n, etc.). Los textos y tablas se deben presentar en el procesador de palabras MS-Word®;las tablas y los diagramas de frecuencia (barras y tortas) originales se deben suministrar en el archivo del documento y también en su original de MS-Excel®; otras figuras, como fotografías sobre papel y dibujos, se pueden enviar en original o escaneadas y ser remitidas en el formato digital de compresión JPG (o JPEG) preferiblemente con una resolución de 600 x 600 dpi (mínimo 300 dpi); es deseable que las fotografías originales sean enviadas como diapositivas. Como norma general, las tablas y figuras sólo se aceptan en blanco y negro; excepcionalmente se incluirán en color cuando sea estrictamente necesario y a juicio del Comité Editorial.

Unidades, abreviaturas y estilo

Se debe utilizar el Sistema Internacional de Unidades (SIU), y aquellas unidades específicas de mayor uso por parte de la comunidad científica. Las unidades combinadas deben usar la forma exponencial. Ejemplo: kg ha^{-1}. El significado de las abreviaturas debe citarse por extenso cuando se mencionan por primera vez en el manuscrito. El estilo de escritura debe ser absolutamente impersonal, en tiempo gramatical pasado para la introducción, los procedimientos y los resultados y presente para la discusión, evitando la conjugación de verbos en primera o tercera persona del singular o el plural.

Los números del uno al nueve se escriben en palabras, excepto cuando incluyen unidades de medida o se mencionan varios números. Ejemplo: “ocho tratamientos”, “3, 7 y 9 lecturas”, “15 kg”. Use cero antes del punto decimal. Para separar números en intervalos de uno o más años, use la letra “a”, y guión para temporadas de crecimiento. Ejemplo: Periodo 2002 a 2005; temporadas de crecimiento 1999-2000, 2000-2001.

Título y autores

El título del artículo no debe incluir abreviaturas y es obligatoria su respectiva traducción al idioma español. En lo posible, el título no debe exceder de 15 palabras y debe reflejar con precisión el contenido del documento. Cuando contenga nombres científicos de especies vegetales o animales, éstos se deben escribir con letra cursiva (itálica) en minúsculas, sólo con mayúsculas la primera letra del género y del clasificador. Debajo del título en inglés se escribe el nombre(s) y apellido(s) de los autores, sin sus respectivos títulos académicos, ni cargos laborales, en una línea horizontal y de acuerdo con su contribución en la investigación y/o preparación del artículo.

Como nota al pie de la primera página, se escribe el título de pregrado, el cargo laboral de los autores, el nombre y la ciudad de ubicación de la entidad a la cual prestan sus servicios o del patrocinador para la realización del trabajo y su respectiva dirección de correo electrónico, indicando el autor de correspondencia. Además, se debe adjuntar un resumen de la hoja de vida de los autores, donde se mencionen los artículos publicados en otras revistas.

Resumen, abstract y palabras claves

El resumen no debe exceder de 250 palabras escritas en un único párrafo. Se debe escribir en inglés y español. Debe contener en forma breve la justificación, los objetivos, los métodos utilizados, los resultados obtenidos más relevantes y las conclusiones. Es obligatorio acompañar el resumen con un máximo de seis palabras clave distintas a las utilizadas en el título. Se aceptan como palabras clave no sólo las palabras simples, sino también términos compuestos hasta de tres palabras. Deben ir escritas en minúsculas y separadas por comas.

Introducción

Puede tener o no título. Define el problema e informa sobre el estado del arte respecto al tema principal del artículo; además, señala las razones que justifican la investigación y plantea los objetivos de la misma. Es obligatorio acompañar los nombres vulgares con el nombre(s) científico(s) y la abreviatura(s) del clasificador en la primera mención dentro del texto. No se deben mencionar marcas de productos, sino su nombre genérico o químico

Materiales y métodos

En este apartado se deben describir en forma clara, concisa y secuencial, los materiales (vegetales, animales, implementos agrícolas o de laboratorio) utilizados en el desarrollo del trabajo; además, se mencionan los aspectos relacionados con la ubicación, preparación y ejecución de los experimentos. Se debe indicar el diseño seleccionado, las variables registradas, las transformaciones hechas a los datos, los modelos estadísticos usados y el nivel de significancia empleado. Evitar detallar procedimientos previamente publicados.

Resultados y discusión

Son la parte central del artículo, deben estar respaldados por métodos y análisis estadísticos apropiados. Se deben presentar de manera lógica, objetiva y secuencial mediante textos, tablas y figuras; estos dos últimos apoyos deben ser fáciles de leer, autoexplicativos y estar siempre citados en el texto. Las tablas se deben elaborar con pocas columnas y renglones. Se debe tener la precaución de incluir el nivel de significancia estadística representado por letras minúsculas del comienzo del alfabeto (a, b, c, d,...), un asterisco simple (*) para $P<0,05$, doble asterisco (**) para $P<0,01$ o triple asterisco (***) para $P<0,001$. Las investigaciones que no siguen un diseño estadístico, deben mostrar la información de manera descriptiva. Use subíndices para modificaciones, reserve superíndices para potencias o notas al pie en tablas y figuras.

La discusión: Se refiere al análisis e interpretación objetiva de los resultados, confrontándolos con los obtenidos en otras investigaciones, o con los hechos o teorías conocidos sobre el tema. Explica los resultados en particular cuando difieren de la hipótesis planteada. Destaca la aplicación práctica o teórica de los resultados obtenidos y

las limitaciones encontradas. Resalta la contribución que se hace a una determinada área del conocimiento y el aporte a la solución del problema que justifica la investigación. Finalmente, proporciona elementos que permitan proponer recomendaciones o lanzar nuevas hipótesis. No se deben hacer afirmaciones que van más allá de lo que los resultados pueden apoyar.

Conclusiones

Son las afirmaciones originadas a partir de los resultados obtenidos, deben ser coherentes con los objetivos planteados y la metodología empleada; además, expresar el aporte al conocimiento en el área temática estudiada y proponer directrices para nuevas investigaciones.

Agradecimientos

Si se considera necesario, se incluyen los agradecimientos o reconocimientos a personas, instituciones, fondos y becas de investigación, que hicieron contribuciones importantes en la concepción, financiación o realización de la investigación.

Formato de citación en el texto

- Se registra la fuente entre paréntesis, el cual debe incluir el apellido del autor y año, con coma entre autor y año. Ejemplo: (Pérez, 1995).

- Si hay más de una fecha se separarán con comas: Ejemplo: (Pérez, 1995, 1998, 2001)

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- 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) …

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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:

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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.
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5.9. Avoid writing about your research in two or more articles from different angles or on different aspects of the research without mentioning the original article.
5.10. Creating various publications based on the same research is considered a type of manipulation.
5.11. If an author wishes to send an article to a journal that is published in a different country or a different language, ask for permission from the editors first.
5.12. When submitting an article, indicate all of the details of the article that were presented in a different language along with the relevant translations.

6. Acknowledging sources

Criteria:

6.1. Authors must cite the publications that had an influence on the determination of the nature of the offered study.
6.2. Privately obtained information cannot be used without the express written consent of the source.
6.3. Republishing tables or figures requires the permission of the author or editor, who must be appropriately cited in the table or figure legend.
6.4. Information obtained through confidential services, such as arbitration articles or subsidy applications, cannot be used without the express written consent of the author of the work involved in said services.

7. Scientific fraud[6]

Criteria:

7.1. Fraud in scientific publications refers to the presentation of false data or conclusions that were not obtained through a rigorous research process.
7.2. The following types of fraud exist for the publication of research results:
a) Fabricating data. Inventing research data and results for later dissemination.
b) Falsification of data. The manipulation of research material, images, data, equipment or processes. Falsification includes the modification or omission of data or results in such a way that the research is not represented in a precise manner. A person may falsify data in order to obtain the desired final results of a study.

Recommendations:

7.3. Before submitting an article, carefully read the editorial and data policies of the journal.
7.4. Never modify, change or omit data intentionally. This includes research material, processes, equipment, tables, citations, and bibliographical references.
7.5. Fabricating and falsifying data constitute grave misconduct because both result in scientific publications that do not precisely reflect the actual observations.
7.6. Authors must appropriately manage the data that supports the research, taking special care in the compilation, production, preservation, analysis and presentation of the data.
7.7. Maintain precise records of the raw data, which must be assessable in case the editors request them after publication of the article.

8. Plagiarism[7]

Criteria:

8.1. Plagiarism is one of the more common types of misconduct in publications; it occurs when an author passes the work of others off as their own without permission, citations, or acknowledgment. Plagiarism can occur in different forms, from literally copying to paraphrasing the work of another person, including data, ideas, concepts, paragraphs, and phrases.
8.2. Plagiarism has different degrees of severity; for example:
a) The quantity of work taken from another person (various lines, paragraphs, pages, or the entire article).
b) What is copied (results, methods, or introduction section).
8.3. Plagiarism, in all of its forms, constitutes unethical behavior and is unacceptable.
8.4. Literal copying is acceptable if the source is indicated and the text is placed in quotation marks.

Recommendations:

8.5. Always remember that it is vital to recognize the work of others (including the work of your assistants or your previous studies).
8.6. Do not reproduce the work of others word for word, in totality or partially, without the permission and recognition of the original source.
8.7. Maintain a record of the sources that are used in the research and where they are used in the article.
8.8. Be sure to accurately acknowledge and cite the original source in your article.
8.9. Even when referencing the source, avoid using the work of others word for word unless it is placed in quotations.
8.10. Paraphrasing is only acceptable if the source is correctly indicated and the source's intended meaning is not changed.
8.11. Use quotations, and cite all of the content that is taken from a previously published source even when using your own words.

9. Fragmentation[8]

Criteria:

9.1.Fragmentation occurs when a large study is divided or segmented into two or more publications.
9.2. As a general rule, as long as the "fragments" of a divided study share the same hypothesis, populations, and methods, this not considered an acceptable practice.
9.3. The same "fragment" can never be published more than one time. Fragmentation can result in distortion of the literature, creating the mistaken belief in readers that the data presented in each fragment (i.e. journal article) are derived from different subject samplings. This not only distorts the "scientific database", but creates repetition that results in a loss of time for editors and evaluators that must work on each article separately. Furthermore, the cited author receives an unfair increase in their number of references.

Recommendations:

9.4. Avoid inappropriately dividing the data of one study into two or more articles.
9.5. When presenting your work, be transparent. Send copies of the manuscripts that are closely related to the manuscript in question, including published, recently submitted and accepted manuscripts.

10. Informed consent

Criteria:

10.1. Studies on patients and volunteers require the approval of the ethics committee.
10.2. The informed consent must be duly documented.
10.3. Permission and waivers must be obtained when an author wishes to include details of a case or other personal information or images of the patients or any other person.
10.4. Special care should be taken when obtaining the consent

of children (especially when a child has special needs or learning disabilities) when their head or face is displayed or when reference is made to the name of an individual or other personal data.

11. Correction of published articles[9]

Criterion:

When an author discovers a significant inexactitude or error in a published article, they must immediately notify the journal and cooperate in the correction process.

References

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