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Presentation of the Content

In the first chapter we present, *Determination of the tomato (Solanum lycopersicum L)'s area foliar through artificial intelligence technique,* by MARTINEZ-RUIZ, Antonio, QUINTANAR-OLGUIN, Juan, PÉREZ-JIMÉNEZ Genaro and FLORES DE LA ROSA Felipe Roberto, with adscription in the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, as a second article we present, *Pre-germination treatment methods on the germination of Caesalpinia coriaria (Jacq.) Willd. and Cassia hintonii Sandwith seeds*, by QUINTANA-CAMARGO, Martín, ROMAN-MIRANDA, María Leonor, AVENDAÑO-LÓPEZ, Adriana Natividad and MORA-SANTACRUZ, Antonio, with adscription in the Centro Nacional de Recursos Genéticos – INIFAP and CUCBA UdeG, as the following article we present, *Interactions between beneficial microorganisms: Endophytic fungi and rhizobacteria*, by GÓMEZ-LUNA, Blanca Estela, MORALES-VARGAS, Adán Topiltzin, DÍAZ-PÉREZ, César and RAMÍREZ-GRANADOS, Juan Carlos, with adscription in the Universidad de Guanajuato, as the following article we present, *Comparison of two protocols for induction of estrus in black belly ewes*, by TABAREZ-ROJAS, Abigail, CRUZ-VARGAS, José Manuel, LAMMOGLIA-VILLAGÓMEZ, Miguel Ángel and CABRERA-NÚÑEZ, Amalia, with adscription in the Universidad Veracruzana.

Content

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Determination of the tomato (*Solanum lycopersicum* L)'s area foliar through artificial intelligence technique

Determinacion del área foliar de jitomate (*Solanum lycopersicum* L) mediante técnica de inteligencia artificial

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Abstract

Studying leaf area (LA) is of vital importance in study of the interception of light, since it has an influence on respiration, dry matter production and directly with consumption of water and nutrients of a plant. Applying artificial neural networks (ANN) as an indirect method to estimate LA has proven to be an accurate tool. In present work, a backpropagation multilayer artificial neural network (ANN) was trained and evaluated to predict LA in a hydroponic tomato crop in a greenhouse. Data collection was carried out by means of a random sampling every 15 days of four plants, which the length and width of each leaf were measured, and by means of an integrator LI-3100, LICOR leaf area was obtained. With this information, the most efficient network structure was searched, the best ANN was trained, validated, and tested. The best neural network structure was obtained with an input variable, a hidden layer with 5 neurons, applying a non-linear activation function of sigmoidal tangent type, where input variable combining characteristic dimensions of leaf was the most efficient.

Machine learning, Artificial neural networks, Simulation models

Resumen

Estudiar el área foliar es de vital importancia en el estudio de la intercepción de la luz, ya que tiene influencia en la respiración, producción de materia seca y directamente con el consumo de agua y nutrimentos de una planta. Aplicar las redes neuronales artificiales (ANN) como método indirecto para estimar el área foliar, ha mostrado ser una herramienta precisa. En el presente trabajo se entrenó y evaluó una red neuronal artificial multicapa (ANN) de retro propagación de los errores, para predecir el área foliar (AF) en un cultivo de jitomate hidropónico en invernadero. La toma de datos se realizó mediante un muestreo aleatorio cada 15 días de cuatro plantas, a las que se midió el largo y ancho de cada hoja, y mediante un integrador LI-3100, LICOR se obtuvo el área foliar. Con esta información se buscó la estructura de red más eficiente, se entrenó, valido y probó la ANN. La mejor estructura de red neuronal se obtuvo con una variable de entrada, una capa oculta con 5 neuronas, aplicando una función de activación no lineal del tipo tangente sigmoidal, donde la variable de entrada combinando las dimensiones características de la hoja resultó la más eficiente.

Aprendizaje de máquina, Redes neuronales artificiales, Modelos de simulación

Citation: MARTINEZ-RUIZ, Antonio, QUINTANAR-OLGUIN, Juan, PÉREZ-JIMÉNEZ Genaro and FLORES-DE LA ROSA Felipe Roberto. Determination of the tomato (*Solanum lycopersicum* L)'s area foliar through artificial intelligence technique. Journal-Agrarian and Natural Resource Economics. 2021. 5-9:1-6.

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Introduction

The leaves are the active frontier for the exchange of carbon and water between plants, canopy and the atmosphere, this green organ helps in feeding plants through photosynthesis and evapotranspiration. The leaf area index (LAI) is a dimensionless variable, it represents a structural attribute of the components of the leaves, it has vital importance in the interception of light which has an influence on respiration, dry matter production, yield and directly with the consumption of water and nutrients, plant growth and development (Firouzabadi *et al*. 2015; Öztürk *et al*., 2019). The LA stimulates the amount of light that enters through the canopy and influences the microclimate; therefore it is used as an indicator of the health of the plant and its development. Therefore, the study of LA or LAI is essential and highly significant in horticultural and agronomic physiological studies. Studies have been carried out that focus on the evaluation of LA using direct and indirect techniques. Although the direct methods are precise, the samplings involve destroying the plants, it is a hard work activity (Colaizzi *et al*., 2017), and complicated as the experimentation area increases. Therefore, nowadays the attention of researchers is focused on looking for indirect methods that avoid the destruction of plants (Hossain *et al*., 2017). Some methods used are, for example, images analysis (Campillo *et al*., 2010), mapping PAR radiation (Zhang *et al*., 2015), use of ceptometers (Mendoza-Pérez *et al*., 2017), others have applied allometric models relating characteristic dimensions of the leaves (Astegiano *et al*., 2001; Colaizzi *et al*., 2017).

In recent years artificial neural networks (ANNs) have been tested within agricultural systems with successful results. Yuan *et al*. (2017) evaluated a neural network that can be used to estimate the LA in soybean crops. On the other hand, Kumar *et al*. (2017) developed an artificial neural network (ANN) based on the characteristic lengths of the leaves as they are; length (L) and width (W) which was compared with regression models, finding greater efficiency of the network compared to regression models. On the other hand, Ahmadian-Moghadam *et al*. (2012) looked for an ANN to calculate the LA of the cucumber crop, finding good adjustments.

Küçükönder *et al*. (2016) used an ANN and analysis of regression techniques to develop the best regression model and conclude that ANN models are an excellent alternative to estimate LA and Shabani *et al*. (2017) used an ANN to estimate the LA of different plants and found that ANN gave acceptable results. The objective of this work was to find the best structure of a multilayer backpropagation artificial neural network (ANN). Training, testing and validation with this network was carried out to predict the LA of a hydroponic tomato crop (*Solanum lycopersicum* L.) in a greenhouse.

Materials and method

Establishment of the experiment

The experiment was carried out in a saw-type greenhouse, with overhead ventilation, located at the Universidad Autónoma Chapingo, with coordinates 19º 29' North latitude, 98º 53 'West longitude, latitude 2240 m. The greenhouse has an N-S orientation with a 700-gauge white plastic cover treated against ultraviolet radiation, with three section (8.5 mx 76 m) a total area of 1938 m^2, three zenith windows, two lateral and two frontals, all with anti-aphid mesh of 25 x 40 threads / in^2. The opening of the vents was carried out in an automated way. The test was carried out in a hydroponic tomato crop (*Solanum lycopersicum* L.), *cv*. "*Rafaelo*", which was germinated on April 7 and transplanted on May 7 with a density of 2.6 plants / m^2, 13-liter pots were used with a combination of tezontle substrate and coconut fiber (70:30). A drip irrigation system with inserted self-compensating drippers was installed, with an injection system and automatic irrigation programming.

Data collection

The LA variable of the crop was measured every 15 days, for this purpose a destructive method was applied, consisting of selecting 4 plants randomly and they were taken to the laboratory, where the characteristic dimensions like length (L) and width (A) of each leaf were measured at the same time with the use of a model leaf area integrator (LI3100, LICOR) the leaf area of each leaf was measured.

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Structure of the neural network

With the data of length, width and leaf area, the best structure of the neural network (Figure 1) of the backpropagation was searched, having as input variables the characteristic dimensions of the leaves; length (L) and width (W) and the different combinations between these variables, length^2 (L^2), width^2 (W^2), length × width (L × W), length^2 × width (L^2 × W) and length × width^2 ($L \times W^2$). The data was organized as follows; For the training of the network, 50% of the data was used, for the test 25% and the validation 25%, for several data (n = 299). A multilayer network of back propagation of the errors (back propagation neural network) was used. To perform the data analysis, they were normalized to a value between 0 - 1 following Equation (1). The procedure consisted of finding the most efficient network that could make the best forecast of the LA variable once trained with a set of data, for which an independent data block (n = 110) was used to perform the validation with the purpose of corroborating the prediction capacity of the network structures. The combination of input with one and two hidden layers were tested, varying the number of neurons per layer from 1 to 7 neurons.

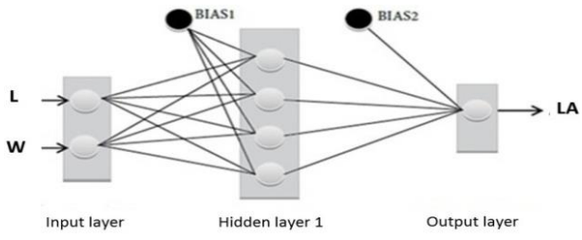

Figure 1 Architecture of a neural network to estimate the leaf area, for two input variables length (L) and width (W), a hidden layer and an output variable (leaf area, LA).

$$Xnorm = \frac{X - Xmin}{Xmax - Xmin} \quad (1)$$

Where $Xnorm$ is the value of the normalized variable (LA), X is the original variable to normalize (LA), $Xmax$ y $Xmin$ are the minimum and maximum original variables (LA). During network training the input values to the network are calculated as follows:

$$Net = \sum_{i=1}^{N} x_i w_i + \quad \theta_i \quad (2)$$

In equation (2), θ_i is the bias value, x_i are the values of the input variable (s), w_i are the values of the weights corresponding to the ith value. To calculate the output values of the network, the sigmoidal function was used as the activation function described in Equation (3).

$$F(net) = \frac{1}{1+e^{(-Net)}} \quad (3)$$

To decide which network structure was the best during training, validation and testing, the correlation coefficient (R) was estimated, to validate the structure of the networks that resulted best, the adjustment statistics were evaluated in addition to the correlation coefficient, the root mean square of error (RMSE) and bias (BIAS) between values measured in the laboratory and those estimated by the neural network.

Results and discussion

As mentioned in the previous section, for the training of the neural network, one and two hidden layers were evaluated, progressively varying the number of neurons per layer, and combining the input variables of the characteristic dimensions of the leaves of tomato cultivation. Where it was found that the most efficient network and with the highest value of the correlation coefficient was for when the structure of the network was formed by a hidden layer with 5 neurons, the adjustments of the measured data versus estimated during the training, validation and test are presented in Figure 2. Where it is evidenced that the input variable that best adjusts the values during the forecast is length × width^2 ($L \times W^2$), considering this combination as an input variable, it is important to mention that the network was also evaluated considering two inputs: the length and the width. However, no good fits were found, so it was decided to use an input variable, and this is given by the different combinations of the characteristic dimensions. The correlation coefficient values in this research (Figure 2) were slightly lower for training (R = 0.98), test (R = 0.97) and all (R = 0.98), found by Küçükönder *et al*. (2016).

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Table 1 shows the results found during the validation of the different structures of the neural network with different neurons, with one and two hidden layers modifying the combinations in the input variable. Where the best fit statistics resulted for when the network was tested with an input variable f (L × W^2) ** and {5} neurons, because the bias values (BIAS) and RMSE were lower and the value of the correlation coefficient (R) was higher in all cases, in descending order, it was for the structure of the network in which the input variable was given by f (L × W) with {7} neurons and finally the third option was when two input variables given by; f (W, L × W^2), with {5} neurons.

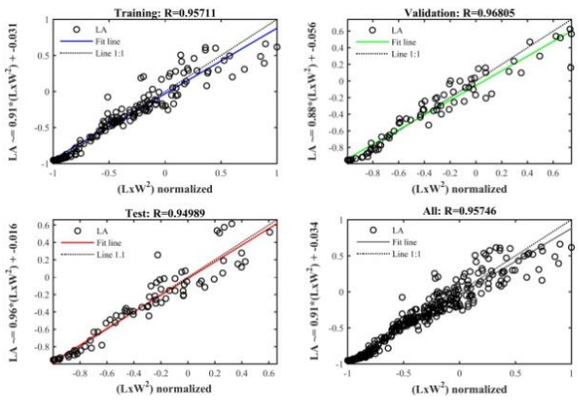

Figure 2 Training, validation and testing of the artificial neural network for the prediction of the leaf area of the tomato crop for a network structure of a hidden layer with five neurons {5}, f (L * W^2)

The predictions for LA given by the best structure of the network were found that the values were similar for the correlation coefficient and very high for the RMSE values (0.11) to those found by Küçükönder *et al*. (2016) for a network of a hidden layer with 4 neurons, in tomato leaves. On the other hand, Ahmadian-Moghadam *et al*. (2012) found that a network of a hidden layer with 2 neurons was the network that best predicted the leaf area in the pepper crop (*Capsicum annuum* L.).

Inputs variables	One hidden layer (neurons)	Statistics of fiting		Two hidden layer (neurons)	Statistics of fiting	
f(L)	{5}	RMSE	89.784	{1 x 7}	RMSE	90.669
		BIAS	-46.845		BIAS	-47.597
		R	0.940		R	0.941
f(W)	{6}	RMSE	69.766	{1 x 6}	RMSE	72.833
		BIAS	-25.450		BIAS	-23.915
		R	0.960		R	0.954
f(L×W)	{7}	RMSE	80.263	{1 x 5}	RMSE	59.084
		BIAS	-13.642		BIAS	-18.168
		R	0.936		R	0.969
f(L×W^2) **	{5}	RMSE	56.890	{1 x 3}	RMSE	56.516
		BIAS	-8.255		BIAS	-9.151
		R	0.967		R	0.968
f(L^2×W)	{6}	RMSE	69.962	{1 x 3}	RMSE	67.619
		BIAS	-29.821		BIAS	-28.532
		R	0.963		R	0.966
f (A, L×W^2)	{5}	RMSE	57.201	{1 x 6}	RMSE	58.447
		BIAS	-13.769		BIAS	-9.046
		R	0.968		R	0.967

Table 1 Statistics of goodness of fit of the validation's performance of the artificial neural network (ANN) for predicting the LA of the tomato crop

When the network was structured using two hidden layers, good adjustments were obtained, although slightly lower than when a single layer was used, the best combination for this case were; in which two input variables f (W, L × W^2) were used with {1 x 6} neurons in the first and second hidden layer, f (L × W^2) with {1 x 3} neurons, In this research work, the evaluations of the network with two hidden layers were carried out just to know if it was possible to find better adjustments since, as is known, as a neural network has more inputs or outputs or more than two hidden layers and number of neurons the complexity of the network structure increases, surely when the problem to be solved is more complex it is justifiable to increase the number of hidden layers and / or the number of neurons. It has been found that using the characteristic lengths improves the prediction of the variable in question, according to the results presented by Astegiano *et al*. (2001) and Küçükönder *et al*. (2016) when evaluating allometric models to evaluate the LA for tomato crop. In Figure 3, the scatter plot of the estimated data of the 6 best configurations of neural networks is shown with an input variable and a hidden layer, which resulted with the best fit in the training, validation and test, in addition the values are presented measured versus values that resulted from the forecast, where it is observed that the data from the neural network that most closely approximates the 45º line (line 1: 1) are those that are similar to the values measured in the laboratory, therefore a lower bias value (BIAS) and those that are concentrated around this 45º line have a lower error (RMSE). Where Figure 3C), 3D) and 3F) correspond to f (L × W^2) ** y {5} neurons, f (L × W) with {7} neurons and f (W, L × W^2), with {5} neurons, respectively.

Öztürk *et al*. (2019) also found excellent fits to using a similar procedure to predict LA in 13 commercial species. While Shabani *et al*. (2017) report surprising results when applying artificial intelligence techniques to predict different crops with different leaf shapes, using the characteristic dimensions of each species.

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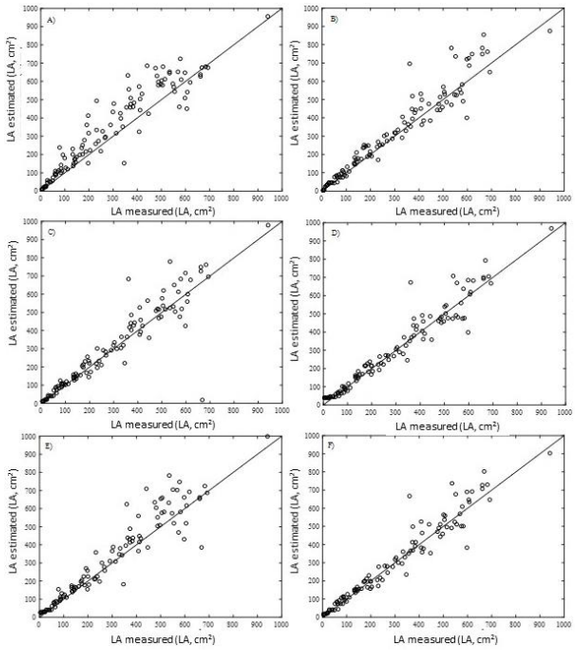

Figure 3 Prediction of the leaf area (FA) of the tomato crop by validating the following configurations of the artificial neural network: A) *f* (Length), B) *f* (Width), C) *f* (Length × Width), D) *f* (Length × Width^2), E) *f* (Length^2 × width), F) *f* (Width, Length × Width^2) and line 1: 1

Conclusions

Artificial neural networks (ANN) are a useful tool to estimate the leaf area of a crop with an acceptable precision, since in most cases they have devices such as leaf area integrators or direct meters of the leaf area index in the field. It is not possible due to how expensive they can be and another disadvantage of these devices is that they are easily decalibrated, and sometimes using them leads to knowing several parameters that are often unknown for a particular crop.

However, it is necessary to test the efficiency of the ANN networks that were better in this work with information from another crop cycle and with other tomato varieties. In the case where the networks with two hidden layers were evaluated and the number of neurons changed, good adjustments were also achieved, however, in order to solve a problem that lacks considerable complexity since it is only interesting to estimate only one subject output variable to a small number of inputs, it is justifiable to use neural networks with a hidden layer.

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Pre-germination treatment methods on the germination of *Caesalpinia coriaria* (Jacq.) Willd. and *Cassia hintonii* Sandwith seeds

Tratamientos pre-germinativos en semillas de *Caesalpinia coriaria* (Jacq.) Willd. y *Cassia hintonii* Sandwith

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Abstract

Recovering degraded ecosystems with native species includes seed management and knowledge of their physiology as a latency mechanism. The objective was to evaluate pre-germination treatments in two native tropical species of the Fabaceae family. The seeds were collected in Colima, Mexico, the germination test was carried out at CUCBA, using 30 seeds per treatment: T1 heat treatment: the seed was immersed in water at a temperature of 80 ° C for three minutes; T2 thermal shock, the seed was immersed in water at a temperature of 80 ° C for three minutes, immediately afterwards in water at a temperature between 0 and 2 ° C for three minutes and T3 (without treatment). The results showed that both species present latency, due to the hard testa characteristic of legumes, the thermal treatments, achieved a higher percentage of germination than the control, in both species. In the emergency speed index (IVE), the heat treatments also generated greater emergency speed. It is concluded that both species need pre-germination treatment to increase their germination percentage, with T1 (temperature at 80 °C) being the one that obtained the best results.

Senna, Cascalote, Dormancy removal

Resumen

Recuperar ecosistemas degradados, con especies nativas incluye el manejo de semillas y conocimiento de su fisiología como mecanismo de latencia. El objetivo fue evaluar tratamientos pre germinativos en dos especies tropicales nativas de la familia Fabaceae. Las semillas se colectaron en Colima, México, la prueba de germinación se realizó en el CUCBA, utilizando 30 semillas por tratamiento: T1 tratamiento térmico: se sumergió la semilla en agua a temperatura de 80 °C durante tres minutos; T2 choque térmico, se sumergió la semilla en agua a temperatura de 80 °C, tres minutos, inmediatamente después en agua a temperatura entre 0 y 2° C tres minutos y T3 (sin tratamiento). Los resultados mostraron que ambas especies presentan latencia, debido a la testa dura característica de las leguminosas, los tratamientos térmicos, lograron un mayor porcentaje de germinación que el testigo, en ambas especies. En el índice de velocidad de emergencia (IVE) los tratamientos térmicos generaron además mayor rapidez de emergencia. Se concluye que ambas especies necesitan tratamientos pres-germinativos para incrementar su porcentaje de germinación siendo el T1 (temperatura a 80 °C) el que obtuvo mejores resultados.

Senna, Cascalote, Rompimiento de latencia

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Introduction

Seed dormancy is an internal condition, which inhibits the germination process, thus it will not take place even under conditions suitable for this process (Benech-Arnold *et al*., 2000). It is of hereditary origin and is considered to be a trait strongly influenced by environmental conditions during seed development and formation (Bewley and Black, 1997; Baskin and Baskin, 2001). This characteristic is the result of physiological adaptations to ensure the survival of the species, as it protects the seeds during times of the year when conditions for germination would not be favourable. Dormancy can be classified according to the mechanism that causes it, and can be of a physical type, characterised by structures that prevent the intrusion of water and even gases, or of a physiological type, when there are mechanisms that act on the metabolism of the embryo, preventing it from developing (Baskin and Baskin, 1998). Propagation and restoration projects often require the introduction of plants obtained from seeds, which may often require prior treatment to eliminate dormancy to germinate successfully (Baskin and Baskin, 1998). Climatic conditions have even been shown to influence both the form (type) and intensity of seed dormancy (Benech-Arnold, 2000).

Caesalpinia coriaria (Jacq.) Willd, of the Family Fabaceae, Subfamily Caesalpinioideae with common name cascalote, dividivi, nacascalotl, nacascul in the state of Guerrero and nacaz-colotl, Xa-gal in the Zapotec language of the Isthmus of Tehuantepec, Oaxaca (Martínez, 1987). It is a tree no taller than 8 m., with scaly bark and very hard reddish wood; alternate, bipinnate compound leaves. Small creamy yellow or pale green flowers, arranged in short, simple or compound racemes. Fruits Indehiscent pods, 3 to 7 cm long, dark brown, curved or curled.

Or curled. Ellipsoid or reniform seeds, 6 to 7 mm long, of a shiny light brown colour. In Mexico it is distributed in the Pacific states from Sinaloa to Chiapas, and is also reported in the state of Mexico, mainly in savannah vegetation and thorny low deciduous forest (Mc Vaugh, 1987; Rzedowski, 1978).

Propagation is by seed (Pintor, 2000). Uses: shade, melliferous, fodder, for wildlife and domestic animals, however, its consumption is mainly whole fruit, as this in the form of flour showed low acceptance by livestock, in a selectivity test with sheep the consumption was 1.3±1.1 g/animal/day (Palma and Román, 2003).

Cassia hintonii Sandwith, of the subfamily Caesalpinioideae, is a tree 8 to 12 m high, with a normal diameter of 20 to 40 cm. Compound paripinnate leaves, 15 to 18 cm long, 15 to 17 pairs of leaflets, dark green and slightly velvety. The flowers are presented in yellow bunches, very showy. It occurs in low deciduous forest and medium sub evergreen forest. It is an important source of nectar and pollen for bees (Quiroz-García et al., 2011). The gum from the seed of dividivi (and other species of the genus Caesalpinia) is used to produce tannins for tanning leather. It is also used to produce dyes and in the manufacture of soaps and toothpaste. The shrub is used as a living fence. The leaves are used to feed livestock. Medicinal properties are attributed to it: the bark and leaves are astringent; the flowers are aromatic and are used against heart ailments and dyspepsia; the roots are used as an antiseptic in ulcerations and against gangrene; in Colombia they prepare a cholesterol-purifying drink. The present study was developed with the aim of removing seed dormancy in *Caesalpinia coriaria* (Jacq.) Willd. and *Cassia hintonii* Sandwith, multipurpose forest species.

Materials and Methods

The seed was collected in the municipality of Ixtlahuacán in the state of Colima, a sample of 30 seeds of each species was used for each pre-treatment, which were: T1) heat treatment, which consisted of seed immersion in water for 3 min at a temperature of 80° C, T2) heat shock, where the seed was subjected to immersion in water at 80°C for 3 min and immediately afterwards placed in water at a temperature of 0 to 2°C, for 3 min, sowing was carried out in germination trays, using peat, under greenhouse conditions. A control (T3) corresponding to untreated seed was included.

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Seedling emergence was counted every 5 days until the end of sowing. In addition to germination, the emergence velocity index (EVI) was also counted. To establish differences in seedling development and emergence under greenhouse conditions, 400 seeds were placed in germination trays using forest soil. The emergence rate coefficient was calculated with the equation proposed by Alm et al:

$$EVI = 1 + \frac{Nj}{Njdj} \quad (1)$$

Where:

EVI = Emergence Velocity Index

Nj = Number of seeds emerged at observation j

dj = Total number of seeds

The values of the speed of emergence coefficient range from 0 to 1.

Results

Figure 1 shows the results of the effect of the pre-treatments, which outperformed the control. Of these, immersion at 80°C was the most effective, since in *Caesalpinia coriaria* seed, 50% more germination was obtained, and in *Cassia hintonii*, this treatment was also the best.

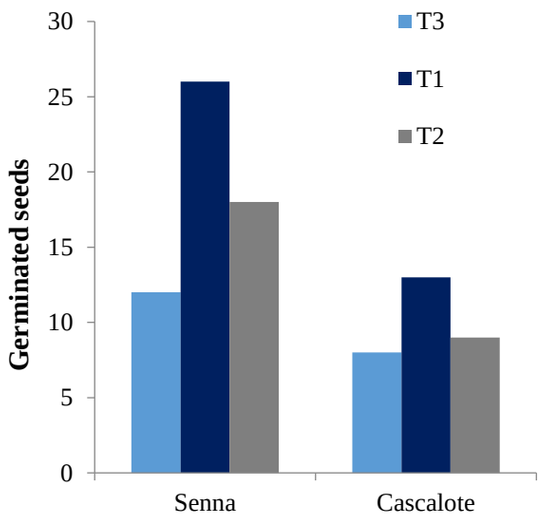

Figure 1. Number of germinated seeds per pre-germination treatment, immersion at 80°C (T1), thermal shock (T2) and control (T3)

Regarding the EVI, table 1 shows that both pre-treatments increased not only the number of seedlings but also the speed of seedling emergence.

The results show, in addition to the convenience of its application, the presence of physical dormancy mechanisms, which can be removed. These results coincide with Reino *et al*, (2011) who reported that stratification in water at 80°C for 2 minutes was more effective in *Crotalaria* sp. seed, demonstrating its effectiveness in eliminating the dormancy imposed by seed coatings (Muñoz *et al*., 2009); and with Atencio, *et al*, 2003, who obtained germination percentages of 90% using hot water at 80°C for 10 min in seed of acacia San Francisco (*Peltophorum pterocarpum*). These results will increase seed germination and thus seedling production for the species under study.

Specie	Days elapsed					TOTAL	EVI
	5	10	15	20	25		
Senna T1	1	13	12			26	**0.078**
Senna T2		11	7			18	**0.052**
Senna T3	1	8	1	1	1	12	**0.042**
Cascalote T1		6	5		2	13	**0.034**
Cascalote T2		6	3			9	**0.03**
Cascalote T3		3	3		2	8	**0.019**

Table 1 Seedling emergence rate under greenhouse conditions

Conclusions

Both species show physical dormancy mechanisms, mainly due to the hard testa characteristic of legumes.

The pre-germinative pre-treatment of immersion in hot water at a temperature of 80°C is the most recommended for seed of both species.

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Interactions between beneficial microorganisms: Endophytic fungi and rhizobacteria

Interacciones entre microorganismos benéficos: Hongos endófitos y rizobacterias

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Abstract

For many years, the obvious benefits of using agrochemicals without restriction were exploited, causing irreversible damage. Currently, it is known that the vast majority of used agrochemicals cause damage to the health of those who handle them, as well as cause deterioration in the soil and contaminate aquifers. For this reason, it is important to create new alternatives that reduce the use of agrochemicals and that fulfil the same functions. The objective of this work was to carry out the interaction between beneficial organisms such as endophytic fungi and plant growth-promoting bacteria, in order to observe positive mutualistic interactions, in order to test beneficial consortia in plant material in future research. In vitro interactions were carried out in PDA medium, inoculum of fungi and bacteria were placed, they were incubated at 28 ° C in a period of time of approximately 8 to 10 days and the photo and type of positive or negative interaction were taken. Around 950 interactions were carried out and of these, 402 were positive interactions. These positive interactions can be used in combination for better plant development.

Beneficial microorganisms, Plants, Environment

Resumen

Durante muchos años se aprovecharon los beneficios evidentes que tenía el utilizar agroquímicos sin restricción, causando daños irreversibles. Actualmente, se sabe que la gran mayoría de agroquímicos usados causan daño a la salud de quien los maneja, al igual que causan deterioro en el suelo y contaminan mantos acuíferos. Por ello, es importante crear nuevas alternativas que reduzcan el uso de agroquímicos y que cumplan con las mismas funciones. El objetivo de este trabajo fue realizar la interacción entre organismos benéficos como hongos endófitos y bacterias promotoras del crecimiento vegetal, con el fin de observar interacciones positivas de tipo mutualista, para en futuras investigaciones probar los consorcios benéficos en material vegetal. Se realizaron las interacciones in vitro en medio PDA, se colocaron inóculos de hongos y bacterias, se incubaron a 28 °C en un periodo de tiempo de aproximadamente 8 a 10 días y se tomó la foto y tipo de interacción positiva o negativa. Se realizaron alrededor de 950 interacciones y de éstas, 402 fueron interacción positiva. Estas interacciones positivas pueden ser utilizadas en combinación para un mejor desarrollo de las plantas.

Microorganismos benéficos, Plantas, Ambiente

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Introduction

Rhizosphere and soil

In a pinch of soil there are millions of bacteria that can transform certain elements into essential nutrients for the survival of living beings present in the rhizosphere. The rhizosphere is of great importance, because in it the exchange of nutrients between the atmosphere and the soil takes place, which involves the microbiota and the plants present in it, that is, ecological-biological processes, and surrounds the system root of the plant. Various authors suggest that the presence of the microbiota is essential for the proper growth of crops and to maintain an ecological balance (Pedraza et al., 2010; Espinosa et al., 2020). In the rhizosphere there are various microorganisms that feed on the exudates of the plant and in turn, strengthen the biological and environmental balance of the soil. The rhizosphere is usually divided into the endorizosphere, which is the intercellular space between the radical tissues colonized by microorganisms, the ectorizosphere, which is the compartment of soil associated with the root up to a distance of 5 mm, and finally, the rhizoplane, which is the interface between the soil and the root (Espinosa et al., 2020).

The concentration of bacteria in the rhizosphere is approximately 10 to 1000 times higher than in bulk soil, but lower than in a laboratory medium. To maintain their beneficial effects in the root environment, bacteria must compete well with other microbes in the rhizosphere for nutrients secreted by the root (Gouda et al., 2018).

There are groups of bacteria capable of transforming atmospheric nitrogen, reducing it into compounds available to other microorganisms and plants. These types of bacteria are often used as parameters of soil quality. Soil quality in ecosystems, whether natural or modified, is evaluated by the ability to maintain or improve plant and animal productivity, water-air quality and habitability, as well as human health (Benjumeda, 2017). In order for the crops and the soil to have a good response to the inoculants, it is necessary to know the interrelation between the inoculant and the native microbiota, since if there is no good interrelation, there may be drawbacks in the cycling of nutrients and organic compounds (Del Puerto et al., 2014).

The participation of bacteria in the transformation depends on their physiological state and enzymatic activity, in addition to the bioavailability of the elements to be transformed and the competition between the microorganisms present in the soil (Pedraza et al., 2010).

Endophytic fungi

Endophytic fungi are microorganisms that inhabit plants without causing apparent symptoms of disease. They are a group of microorganisms that can belong to the genus *Ascomycota*, *Basidiomycota*, *Zygomycota* and *Oomycota*. They manage to inhabit the leaves, stems, flowers or roots of a plant and the location of the fungus in the plant depends on the species. Generally, the concept of endophytic association is associated with mycorrhizae, since they describe a mutualistic relationship, however, endophytic fungi that do not form mycorrhizae have been found and have the same benefits (Ordóñez et al., 2012).

The relationship they form with the plant can be mutualistic, neutral or antagonistic. The classification of these fungi depends on where they are located in the host plant. One of the most common classifications is: claviciptacean endophytes and non-claviciptaceous endophytes (Jambon et al., 2018).

The presence of endophytic fungi was documented in 1898, when they observed that certain animals after consuming grasses presented symptoms of intoxication, but it was until 1977 that Bacon related the presence of *Neotyphodium coenophialum* with summer syndrome (Sánchez et al., 2013). In 1988 Clay proposed the mutualistic interaction that exists between certain fungi and grasses.

The relationship between an endophytic fungus and its host plant is related to the production of virulence factors and defense metabolites produced by the plant either by biochemical or mechanical means, environmental factors and the development stages of both organisms (Frey et al., 2011). When there is balance between the factors already mentioned, an endophytic relationship occurs. However, if the plant is in senescence or under stress, the fungus will be detected as a pathogen, therefore, it will manage to infect the plant and thus produce symptoms (Sánchez et al., 2013).

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The relationship that exists between the host and the host is of great interest, since the fungus is capable of producing bioactive metabolites, as well as modifying the defense mechanisms of its host, allowing and increasing the survival of both organisms (Gamboa, 2006).

Endophytic fungi can protect the plant against biotic and abiotic factors, in addition to producing allelopathic metabolites, this means that it prevents the growth of other microorganisms around it. The protection mechanisms are divided into three, direct, indirect and ecological (Sánchez et al., 2013). The direct protection mechanism is characterized by the production of enzymes or secondary metabolites, on the other hand, the indirect mechanism has the ability to induce the chemical and physiological defense mechanisms of the plant. The ecological protection mechanism is through predation or hyperparasitism (Sánchez et al., 2013).

Fungi contribute to the mineralization of carbon in the soil, they also have an antagonistic role against phytopathogenic fungi. They generally degrade compounds formed by cellulose since the nitrogen requirement is usually low (Sánchez et al., 2013). Yeasts are a group of microorganisms belonging to the fungi kingdom, the main genera used are *Saccharomyces* and *Candida*. Their main function as beneficial microorganisms are the production of enzymes and hormones that can be used by lactic acid bacteria.

In addition, they have a wide spectrum in their carbon sources and are not capable of assimilating nitrites or nitrates as a nitrogen source and their phosphorus sources are in the form of sulfates (Ordóñez et al., 2012). The main fermenting fungi are *Aspergillus oryzae* (it has uses in western cuisine as a fermenter of various cereals and legumes, it is a filamentous, aerobic fungus), *Penicillum* sp (they have an important function in the degradation of cellulose and lignin, in addition, it has a good adaptation in acidic environments and with low water levels) and *Trichoderma* sp (produces various enzymatic compounds capable of degrading organic matter, it can also be found almost anywhere) (Morocho & Leiva, 2019).

Plant growth promoting bacteria

Plant growth promoting bacteria (PGPR) can be aerobic anaerobic or facultative anaerobic (Loredo et al., 2004). They are found in the rhizosphere and are microorganisms that have the ability to stimulate the growth of certain plant species, they are characterized by their efficiency by fixing nitrogen, solubilizing phosphates (organic and inorganic), producing indole compounds, as well as in the decomposition of crop residues, mineralization of organic matter and immobilization of mineral nutrients (Espinosa et al., 2020). PGPRs modify the physiology of plants and the nutritional properties of the soil. In addition, it has been shown that they increase the absorption of compounds such as calcium, potassium, iron, copper and zinc, through the production of organic acids by the plant and the decrease in pH by PGPR (Loredo et al., 2004; Espinosa et al., 2020).

The mechanisms of the PGPR can be direct or indirect, the direct effects are the fixation of atmospheric nitrogen, production and synthesis of siderophores, solubilization of minerals, the synthesis of phytohormones, as well as the production of ACC deaminase, antibiotics, enzymes, competition, hydrogen cyanide, RSI, and quorum extinction (Oluwaseyi et al., 2017). The indirect mechanisms of PGPR are through the inhibition of the growth of other microorganisms, generally pathogens. That is, the bio-control of phytopathogens mainly by the production of antibiotics and iron reduction. Currently, the application of these bacteria can be through inoculation of seeds, substrates, seedlings, foliage and fruits (Pedraza, et al., 2010; Virgen, 2011).

Some authors such as Gouda (2018), have classified PGPR as growth promoting rhizobacteria (ePGPR) and intracellular plant growth promoting rhizobacteria (iPGPB). Within the ePGPR are those that inhabit the rhizosphere (in the rhizoplane) or in the spaces between the cells of the root cortex. The main ePGPRs are *Azotobacter*, *Serratia*, *Azospirillum*, *Bacillus*, *Agrobacterium*, *Flavobacterium*, *Arthrobacter*, *Micrococcous*, *Pseudomonas* and *Burkholderia*. On the other hand, iPGPBs inhabit nodular structures, the main endophytic bacteria are Allorhizobium, Bradyrhizobium, *Mesorhizobium* and *Rhizobium* (Gouda et al., 2018).

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In regions where climatic conditions have high humidity, the soil tends to have an acid pH, due to the leaching of cations. This type of soil tends to produce chemical compounds like iron and aluminum oxide. In regions with a dry climate, the soil tends to have high concentrations of alkaline cations, that is, a basic pH (Scherlach et al., 2013). Therefore, pH plays a very important role, since in the case of having acidic soils, plant growth, production, yield, population and microbial activity, both beneficial and pathogens, can be affected. As well as the availability of nutrients in the soil (Espinosa et al., 2020).

The main function of *Actinomycetes* consists in the solubilization of the cell wall or components of plants, insects or fungi. Therefore, they are of great help in composting and improving soil quality (Pérez et al., 2015). The main genera of *Actinomycetes* are *Streptomyces*, some of these species have important roles in biological control, since they produce hydrolytic enzymes and thus can inhibit the growth of phytopathogenic fungi (Morocho & Leiva, 2019). Bacteria of the genus *Pseudomona* promote plant nutrition, regulate hormone levels and the expression of genes related to growth, as well as the induction of antioxidants and osmolytes. In general, rhizobacteria improve the photosynthetic rate due to the excellent assimilation of CO_2 and due to its function at the photochemical level in photosystems (Pérez et al., 2015; Morocho & Leiva, 2019).

Methodology

Obtaining strains of bacteria and fungi

From existing collections of endophytic fungi and plant growth-promoting bacteria, a duplicate of the 24 fungal strains and 52 bacterial strains was generated. Obtaining the duplicates of fungi was done by using papers containing the inoculum, subsequently, under sterile conditions, the inoculum was placed in a Petri dish with PDA medium. The growth conditions for the fungi were: a temperature of 28 ° C for a period of 7 days in an incubator, avoiding the change in temperature and controlling the surrounding light. Finally, they were stored in a refrigerator at a temperature of 18 ° C.

The bacteria were obtained from an existing collection; the inoculum was contained in Petri dishes with PDA medium.

To generate the copies of the PGPR, the inoculum was taken and seeded in a potato dextrose medium, contained in sterile Petri dishes. The growth and storage conditions were similar to those of the fungi, with the difference that the growth period was 1 to 3 days, with an approximate temperature of 28 ° C.

Sowing and conditions of interactions

About 950 interactions were made in vitro, with a duplicate of each. For this, potato dextrose medium (PDA) was prepared and subsequently emptied into sterile Petri dishes. The boxes with PDA medium were used to place inoculum of fungi and bacteria. Finally, a fungi was placed in the center of the box and 4 bacteria around it, as shown in the figure 1.

Once the bacteria and fungi had been planted in the boxes prepared with PDA, they were sealed with a plastic film and labeled, later they were stored in a room with little light, at an approximate temperature of 24 ° C in a period of time approximately 8 to 10 days.

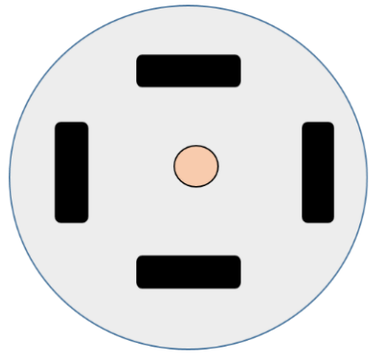

Figure 1 The interaction between 1 fungus - 4 bacteria

Take picture

Once the incubation time of the interactions had passed, their analysis was carried out. Once the interactions obtained were observed, photos were taken of the boxes that contained the interactions, for this, two backgrounds were used, a black and a white background. To take the photo a Sony camera of 32 mega pixels were used. It is important to take into account the background in which the interactions will be placed for the image taking, because the different strains of fungi and bacteria have different colorations, therefore, to facilitate the visualization of the interactions and provide an accurate analysis, it is they selected two backgrounds (black and white).

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In the present work, genera of fungi *Aspergillus*, *Fusarium*, *Penicillium* and other fungi that are not yet characterized were used. These genera can pigment the medium with red, purple, green and black colors.

Results

The interactions occur through signals produced by being in contact with certain volatile compounds or that diffuse in the medium. However, the invasion of the fungus by means of spores or mycelia towards the bacterial strain present in the medium was considered a positive interaction. That is, if the development of the fungus was allowed from the center of the Petri dish towards the walls. In several cases, the fungus managed to colonize various parts of the Petri dish, until it reached the bacteria, covering it completely or partially.

On the other hand, in some cases the bacteria generated larger colonies preventing the invasion of the fungus, which was considered a negative interaction, since the inhibition of both microorganisms was observed. In some cases, the interaction of the fungus is perceived above and below the bacteria, with the generation of mycelia and / or spores. It should be noted that generally the mycelium was observed more clearly on the black background, while on the white background it was possible to visualize the sporulation present in the medium.

Figure 2 shows the growth of fungi and the interactions of fungi and bacteria. A total of 932 interactions were carried out and of these 402 were positive, that is, they could form consortia.

The strains used in in vitro interactions have been studied and their effectiveness in terms of benefits in the growth of plant material has been proven. In this study, the interaction of the strains as a mutualistic community was observed and so far they have not been applied in plant models, therefore, the effectiveness of each of the interactions obtained has not been evaluated. However, after having information on the mutualistic interaction of these microorganisms individually, that is, in a fungus-plant interaction, or plant bacteria, it is expected that by being in a tripartite interaction (fungus-bacteria-plant) the development and the benefits are tangible.

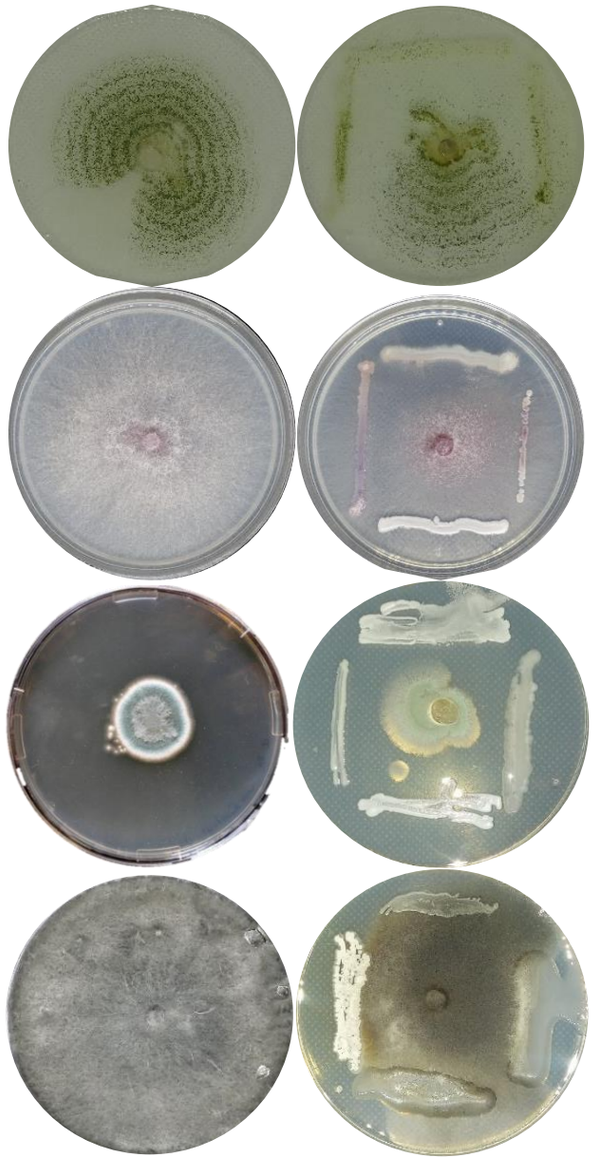

Figure 2. Fungus *Aspergillus*, *Fusarium*, *Penicillum* and H-01 and Interactions positive whit bacterial strain

Conclusions

The interactions obtained can have an important place in the market or in research, since not only can they function as an alternative to agrochemicals, but also have diverse functions such as bioremediation and increase in the quality of the ground and with it brings benefit, after benefit. There is still a long way to go in terms of the application of plant material, among other studies. For the application of consortia, it is necessary to take into account that all microorganism communities have a peculiar microhabitat that depends on the host species, type of root and the composition of the root exudates. These factors influence the production of various volatile compounds, which involves the growth of plants.

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Comparison of two protocols for induction of estrus in black belly ewes

Comparación de dos protocolos de inducción del estro en ovejas de la raza black belly

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Abstract

The aim was to evaluate two protocols for the induction of estrus in hair ewes on the presentation of estrus and fertility. Forty-six ewes of the Black Belly breed were used, the female was divided into two groups: 1) protocol 12 days (P12) and 2) protocol 9 days (P9). P12 sheep were placed with an intravaginal sponge and 400 IU eCG was applied two days before device removal. P9 sheep were sponge inserted, 400 IU eCG was applied two days before device removal, and cloprostenol 250 mcg was applied at the time of removal. All ewes inseminated by laparoscopy. The pregnancy diagnosis was made by ultrasound 48 days after artificial insemination. 100% of the sheep were in estrus. Fertility was 78.3% in P9 ewes and 91.3% in P12 ewes, with no differences between the groups (P>0.05). In conclusion, the use of P9 for the induction of estrus is as effective as P12 in the presentation of estrus and fertility percentage at 48 days.

Ewes, Estrus induction, Fertility

Resumen

El objetivo fue valorar dos protocolos de inducción del estro en ovejas de pelo sobre la presentación del celo y la fertilidad. Se utilizaron 46 ovejas de la raza Black Belly, las hembras fueron divididas en dos grupos: 1) protocolo con duración de 12 días (P12) y 2) protocolo con duración de 9 días (P9). A las ovejas del P12 se les colocó una esponja intravaginal y se aplicaron 400 UI de eCG dos días antes del retiro del dispositivo. A las ovejas del P9 se les insertó una esponja, se aplicaron 400 UI de eCG dos días antes del retiro del dispositivo y se aplicaron 250 mcg de cloprostenol al momento del retiro. Todas las ovejas fueron inseminadas por laparoscopía. El diagnóstico de gestación se realizó mediante ecografía 48 días después de la inseminación artificial. El 100% de las ovejas presentaron celo. La fertilidad fue de 78.3% en las ovejas del P9 y 91.3% en las ovejas del P12, sin observar diferencias entre los grupos (P>0.05). En conclusión, el uso de P9 para la inducción del estro es tan efectivo como el P12 en la presentación del celo y porcentaje de fertilidad a 48 días.

Ovejas, Inducción del estro, Fertilidad

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1. Introduction

Reproductive biotechnologies such as synchronization and / or induction of estrus and artificial insemination are tools that allow accelerated genetic progress, in addition to allowing better reproductive control of the herd, especially to better control the calving season, cover in the anestrous season, group animals into homogeneous lots, schedule weaning and sell animals by batch.

On the other hand, profitability can be substantially improved since synchronization and / or induction protocols increase female prolificacy and the number of parturitions per female per year increases.

The synchronization and / or induction of estrus can be carried out by different methods, these can be natural or hormonal. The most widely used hormonal-type estrus induction or synchronization protocols are based on polypropylene sponges impregnated with progestogens and silicone devices impregnated with natural progesterone. There are also other types of hormonal products that are administered parenterally, such as equine chorionic gonadotropin (eCG), gonadotropin-releasing hormone (GnRH) and prostaglandin F2α (PGFα) and their synthetic analogues, which when used in combination with progesterone and its analogues also synchronize ovulation during the time of year when fertility is decreased or the regression of the previously existing or newly formed corpus luteum (Tabarez and Grajales, 2020).

In recent years, short-term treatments have been proposed, being able to reduce exposure to progesterone to only 5-7 days (Menchaca et al., 2007). Therefore, the objective was to compare the effect of two protocols of estrus induction (12 days vs. 9 days duration) in hairy sheep of the Black Belly breed on the presentation of estrus and fertility at 48 days.

2. Material and methods

The research was carried out during a positive photoperiod (March to April) in a Livestock Production Unit located in the municipality of Tihuatlán, Veracruz, which is located in the north of the state at coordinates 18 ° 27 'north latitude and 96 ° 21 'west longitude at a height of 60 meters above sea level.

Its climate is warm-regular, with an annual average temperature of 22 ° C; its average annual rainfall is 1,076.2 mm (INEGI, 2015).

46 second parturition females of the Black Belly breed were used, with body condition 3 on a scale of 0 to 5 (Russel et al., 1969), the females were randomly divided into two groups: group P12, estrus induction protocol with duration of 12 days (n = 23) and group P9, protocol of induction of estrus with duration of 9 days (n = 23).

In the sheep of group P12, on day 0 a polyurethane sponge containing 20 mg of cronolone (Chronogest CR®) was placed intravaginally, remaining in the insertion site for 12 days, on day 10 they were applied intramuscular 400 IU of equine chorionic gonadotropin (eCG; Novormon 5000 ®) and the sponge was removed on day 12.

In group P9 sheep, on day 0 the sponge was inserted intravaginally, remaining in the insertion site for 9 days, on day 7 was applied intramuscularly 400 IU of eCG and on day 9 it was performed removal of the sponge and 250 mcg of synthetic prostaglandin cloprostenol (Celosil ®) were applied intramuscularly.

The estrus detection of all the ewes was carried out 24 hours after the removal of the sponge using a ram with penis deviation, the detection was carried out twice a day (in the morning and in the afternoon), a female was considered in estrus when he allowed the riding of the ram. All ewes were inseminated at a fixed time (50 hours after removal of the sponge), artificial insemination (AI) was performed by laparoscopy with thawed semen.

The pregnancy diagnosis was made 48 days after AI by transrectal ultrasound. All the sheep were fed daily with 2.7 kg of wet orange silage, 2 kg of insurgent grass (*Brachiaria brizantha*) hay and 0.5 kg of balanced feed with 18% crude protein. The water and mineral salts were provided freely available.

The statistical analysis of the variable hours of presentation of estrus was performed with the Student's t test for independent samples and the fertility variable, evaluated as a percentage of gestation at 48 days, was analyzed using the Chi-square test, using the SPSS statistical package 24 for MAC (IBM SPSS, 2016).

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3. Results

Ewes in both the 9-day and 12-day protocols responded to estrus induction, 100% of the ewes in both groups manifested estrus. Regarding the time in which they presented estrus after removal of the intravaginal sponge, the ewes of the 12-day protocol presented it 30.31 ± 0.05 hours after the removal of the sponge and the ewes of the 9-day protocol presented estrus at 30.29 ± 0.02 hours, without observing differences between the groups (Pα 0.05; Table 1).

Estrus induction protocol	Hours of presentation of estrus
12 days	30.31±0.05
9 days	30.29±0.02

Table 1 Hours of presentation of estrus (mean ±standard error) after removal of the intravaginal sponge in sheep with estrus induction using a protocol of 12 days vs. 9 days.

The fertility at 48 days in the sheep of the 12-day estrus induction protocol was 91.3% and in the sheep of the 9-day protocol it was 78.3%, without observing statistically significant differences between the groups (P> 0.05; Table 2).

Estrus induction protocol	Fertility percentage
12 days	91.3 (21/23)
9 days	78.3 (18/23)

Table 2 Percentage of fertility in ewes with induction of estrus using a protocol of 12 days vs. 9 days.

4. Discussion

In the present study, 100% of the ewes exhibited estrus, both in the 9-day estrus induction protocol group and in the 12-day protocol group, unlike Farfán et al. (2009), who when comparing a 12-day and a 6-day synchronization protocol using a combination of parenteral PGF2α and intravaginal sponges impregnated with 50 mg of medroxyprogesterone, obtained only 100% of sheep in heat in the 12-day treatment , while in the 6-day protocol only 85.7% presented it. Similarly, Balcázar, (2013) obtained a percentage of estrus synchronization in Dorper sheep that was lower than the present study, after comparing two synchronization schemes, a 5-day protocol and a 12-day protocol, using PGF2α and new CIDR in both groups. impregnated with 0.3 g of natural progesterone and 200 IU of eCG to the sheep of the short protocol.

The percentage of synchronization was 94.7% in the group that received a 5-day treatment and 81.6% for the group with a 12-day treatment. The differences observed in the aforementioned studies were not statistically significant, this means that the short-term regimens with natural or synthetic progesterone are as effective for the synchronization or induction of estrus in sheep, as the long regimens.

Regarding the time of presentation of estrus after removal of the intravaginal sponge, no statistically significant differences were observed between the two protocols, P12 and P9, the ewes of both groups showed the estrus during the first 31 hours. Coinciding with Farfán et al. (2009), who also found no significant differences for said variable between sheep synchronized with PGF2α and a progestogen for 12 days (58.0 ± 16.09 h) and sheep synchronized with PGF2α and progestogen for 6 days (56.4 ± 6.76 h), although the time in which they presented estrus was higher than that observed in the present study.

The pregnancy percentage obtained was lower in the ewes of the 9-day protocol (78.3%) compared to the ewes of the 12-day protocol (91.3%), however, the difference (13%) was not statistically significant, coinciding with Farfán et al. (2009), who also did not observe statistically significant differences between the short synchronization protocol (6 days) and the long protocol (12 days), obtaining a conception rate of 75% in the short treatment and 71.42% in the long treatment. In contrast, Balcázar, (2013) obtained a significantly higher pregnancy percentage in the group of sheep of the 5-day protocol (71.1%) compared to the group of sheep of the 12-day protocol (31.6%). Similarly, Raso (2004), obtained a higher percentage of pregnancy in the short protocol of 6 days (91%) than in the long protocol of 12 days (62.5%), when synchronizing Merino sheep using intravaginal sponges impregnated with Medroxyprogesterone Acetate plus 300 IU of PMSG. Menchaca et al. (2007) point out that the fundamental objective of short protocols is to avoid sublethal progesterone concentrations for prolonged periods and to ensure adequate levels that allow follicular turnover and ovulation of fertile oocytes.

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5. Conclusion

The use of a 9-day protocol for the induction of estrus with intravaginal sponges impregnated with 20 mg of cronolone was as effective as the 12-day protocol in the presentation of estrus, time of presentation of estrus after removal of the device and percentage of fertility.

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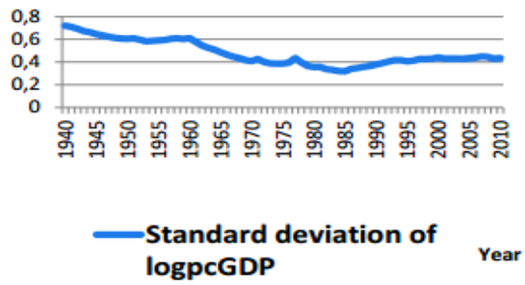

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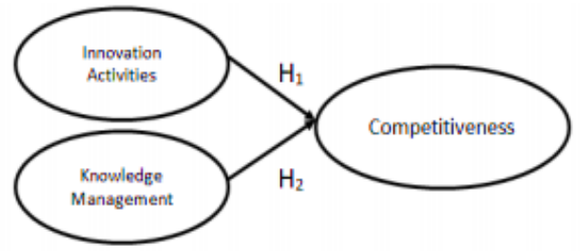

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