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Presentation of content

In the fist article we present, *Exploitation of the genetic variance in maize (Zea mays L.) populations for the mexican tropic*, by SIERRA-MACIAS, Mauro, RÍOS-ISIDRO, Clara, GÓMEZ-MONTIEL, Noel Orlando and BARRÓN-FREYRE, Sabel, with adscription in the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, in the next article we present, *Antibacterial activity of orégano (Lippia graveolens) essential oil against Staphylococcus aureus in vitro*, by VARGAS-MONTER, Jorge, SIFUENTES-SAUCEDO, Diana María, ZARAGOZA-BASTIDA, Adrián and NIETO-AQUINO, Rafael, with adscription in the Universidad Politécnica de Francisco I. Madero, Universidad Autónoma del Estado de Hidalgo, Tecnologico Nacional de México. Campus Ciudad Valles, in the next article we present, *Response of cotton (Gossypium hirsutum L.) seeding the ultra-narrow grooves and high population density*, by MARTINEZ-LARA, Filiberto, CHINO-CANTOR, Araceli, MARTÍNEZ-SÁNCHEZ, Itzcóatl and CIPRIANO-ANASTASIO, Juan, with adscription in the Universidad Politécnica de Francisco I. Madero, in the netx article we present, *Use of rapid test glycoproteins associated with pregnancy and ultrasound in early diagnosis of pregnancy in cow*, by GARCÍA-GOZÁLEZ, Daniela, ALARCÓN-ZAPATA, Marco Antonio, GARCEZ-MERCADO, Nora and TABAREZ-ROJAS, Abigail, with adscription in the Universidad Veracruzana.

Content

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Exploitation of the genetic variance in maize (*Zea mays* L.) populations for the mexican tropic

Aprovechamiento de la varianza genética en poblaciones de maíz (*Zea mays* L.) para el trópico mexicano

SIERRA-MACIAS, Mauro†*, RÍOS-ISIDRO, Clara, GÓMEZ-MONTIEL, Noel Orlando and BARRÓN-FREYRE, Sabel

Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, INIFAP

ID 1st Author: *Mauro Sierra-Macías* / **ORC ID:** 0000-0001-6476-2192, **CVU CONAHCYT ID:** 5116

ID 1st Co-author: *Clara Ríos-Isidro* / **ORC ID:** 0000-0003-2148-3745

ID 2nd Co-author: *Noel Orlando Gómez-Montiel* / **CVU CONAHCYT ID:** 5945

ID 3rd Co-author: *Sabel Barron Freyre* / **CVU CONAHCYT ID:** 459260

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Abstract

VS-536, The most used maize variety in the southeast of México, present favorable plant and ear traits and adaptation to the tropical conditions. The objectives of this research were to exploit the genetic variance present in maize populations, particulary in the improved version of VS-536. In mass selection is exploited the additive portion of the genetic variance. In varietal crosses of maize formed and evaluated during 2016 to 2023, there was found int the best hybrids, the presence of the VS-536 variety as parent. It suggest that an important portion of the genetic variance correspond to dominance and overdominance gene action and they can be exploited through varietal crosses as SINT4BxVS-536 and V-537CxVS-536; These crosses registered the highest grain yield across the 10 environments in Veracruz and Tabasco states of 6.84 and 6.34 t ha^{-1} statistically similar to H-520, used as check, that registered 6.73 t ha^{-1}; Heterosis values with respect to the best parent were 15.80 and 11.21% for each cross respectively. The varietal cross SINT4BxVS-536 present short plant and ear height, tolerant to lodging, good plant and ear aspect and sanity, the leaves above the ear are in semierect position, excellent husk cover, the grain is white and present semident texture.

Gene action, *Zea mays* L., *Aditivity, overdominance*

Resumen

VS-536, variedad de maíz de mayor uso en el sureste mexicano, presenta características favorables de planta y de mazorca y adaptación a las condiciones del trópico. Los objetivos de este trabajo fueron aprovechar la varianza genética presente en poblaciones de maíz, particularmente en la versión mejorada de VS-536. La selección masal aprovecha la porción aditiva de la varianza genética. De cruzamientos varietales de maíz formados y evaluados durante 2016 al 2023, se encontró un grupo sobresaliente en el que se observó la presencia de VS-536 como progenitor. Lo anterior sugiere una porción importante de genes con tipo de acción génica dominancia o sobredominancia que pueden ser aprovechados en cruzas varietales como SINT4BxVS-536 y V-537CxVS-536; Estas cruzas registraron rendimientos a través de 10 ambientes en Veracruz y Tabasco de 6.84 y 6.34 t ha^{-1} estadísticamente similar al testigo H-520, mismo que registró 6.73 t ha^{-1}; La heterosis con respecto al mejor progenitor fue de 15.80 y 11.21% para cada cruza respectivamente. La cruza varietal SINT4BxVS-536 presenta altura de planta y mazorca baja, tolerante al acame, buen aspecto y sanidad de planta y de mazorca y las hojas arriba de la mazorca en posición semierecta, excelente cobertura de la mazorca, grano de color blanco y textura semidentada.

Acción génica, *Zea mays* L., Aditividad, sobredominancia

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* Author Correspondence (E-mail: sierra.mauro@inifap.gob.mx; mauro_s55@hotmail.com)
† Researcher contributing as first author.

Introduction

In México Maize is the most important crop because of it is part of the diet for human consumption, the sown area and generate 36% of the agriculture production value. During 2022, there were sown in México, 7.47 million of hectares with maize, which of them, 6.904, were for grain production, with an average in yield of 3.90 t ha^{-1}, and a total production of 26.55 million tons, which of them 19.35 million tons are utilized in different ways through the direct consume for human consumption; Besides, during 2022, there were imported 17.40 million tons of yellow grain for animal feed industry and an apparent *per cápita* consume of 338.10 kg; (SIAP, 2022). Improved seeds are the most important input in corn production, they represent the genetic yield potential and quality production (Sierra *et al.,* 2016).

In the humid tropic in México, at the same year there were sown 2.8 million of de hectares with maize, which of them, one million are included in agronomic provinces of good and very good productivity, and 91 thousand hectares under irrigation conditions, where is recommended the improved seed of synthetic maize varieties and hybrids (SIAP, 2022; Sierra *et al*., 2019). In the maize breeding program of Cotaxtla experimental station, INIFAP, there have been generated hybrids and synthetic maize varieties, which expressed good yield and favorable agronomic characteristics through the tropical region in the southeast of México, but above all, they have been adopted by maize farmers (Sierra *et al.,* 2019).

The synthetic maize varieties is the best way of joining the good *per se* grain yield of inbred lines and their general combining ability in generating varieties adapted to the humid tropic in México. This kind of germplasm can be used by farmers for several seasons of sown without affecting the grain yield and is easier and profitable the commercial seed production, (Márquez *et al.,* 1983; Reyes 1985; Andrés *et al.,* 2017).

Márquez *et al.,* (1983) define a synthetic maize variety those that can be maintaining by open pollination, after joining by hybridization in all combinations among one number of selected genotypes The synthetic maize varieties have been proposed as an alternative in using of hybrid (Coutiño *et al.,* 2017).

The synthetic maize varieties present an additive portion of the genetic variance, that can be exploited trough recurrent selection. However, an important portion of the genetic variance correspond to dominance and overdominance gene action that can be exploited through the heterosis in varietal crosses (Sierra *et al.,* 2019; Márquez 2014). High heterosis values suggest that exist genetic divergence between the parental varieties (Reyes, 1985; Márquez 2014; Córdova *et al.,* 2007; Sierra *et al.,* 2004; Ramírez *et al.,* 2019; Palemón *et al.,* 2012; Velasco *et al.,* 2019; Trachsel *et al.,* 2016; Gómez *et al.,* 2017).

Márquez (1974), in his contribution, the problem of the Genetic Environment Interaction in plant breeding, he wrote, F= G+E+GE,

Where:

F= Phenotype

G= Genotype

GE= Genetic Environment Interaction

According with this, if we eliminate or reduce the environment effect, automatically is eliminated the interaction and the formula is expressed as follow: F=G

In this way, is more efficient plant or family selection for each one recurrent selection method. The ways more efficient for eliminating the environmental effect are the stratification of selection plots and consider plants with complete competence or using experimental designs with replications than permit to reduce the experimental error or environmental variance.
Besides, a condition for getting genetic response to selection is that exist genetic variability in maize populations (Sierra *et al.,* 2019).

Varietal hybrids in maize represent an alternative in maize commercial production due to heterosis that result of crossing two open pollinating maize varieties as parents; Besides another advantage in this kind of hybrids are that only two parents for maintaining and is easier and profitable the commercial seed production (Sierra *et al.,* 2018; Sierra *et al.,* 2016; Reyes, 1985; Tadeo *et al.,* 2021; López *et al.,* 2021; Palemón *et al.,* 2012).

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The objectives of this research were: a) To practice mass selection in the synthetic maize varieties VS-536, V-537C and SINT4B for breeding in yield and agronomic traits, b) To know the yield and the heterosis for yield with respect to the best parent in varietal crosses and c) To know the agronomic traits for the best maize varietal crosses, using the synthetic variety VS-536 as a male parent.

Materials and Methods

Localization. The mass selection plots of the maize varieties VS-536, V-537C and Sint 4B and the plot for getting the varietal crosses, were carried out in Cotaxtla experimental station in Veracruz, which belongs to INIFAP, México and is located at the km 34 through the public road from Veracruz-Córdoba in the municipality of Medellín de Bravo, Ver., in the 18º 56' North Latitude and 96º 11' West longitude and altitude of 15 masl. The evaluation of the maize hybrids was carried out in Cotaxtla Experimental Station and CBTA 84 in Carlos A. Carrillo municipality in Veracruz, and Huimanguillo in Tabasco, The clime condition is Aw1(w), Aw2 y Am, for each location, respectively; According with the clime classification described by Köppen and modified by García (2004), correspond to humid and subhumid warm conditions with average annual temperature of 25 ºC and annual precipitation of 1400 mm, distributed from june to november with a dry season from December to May. The soil is Vertisol, from alluvial origin, deep, with medium texture troughout the profile, slope less than 1% and good drainage and slightly acid pH (6.6) (INEGI, 2020).

Germplasm used. The germplasm used in the present research, belongs to the Tuxpeño race and they were the synthetic maize varieties VS-536, V-537C and SINT 4B on wich there were practiced four seasons of mass selection for breeding in yield and agronomic traits, and varietal maize crosses formed with experimental synthetic varieties wich of them belongs to tuxpeño race. There were evaluated 28 genotypes, wich of them 20 were varietal crosses, five experimental synthetics, the commercial varieties VS-536 and V-537C and the commercial hybrid H-520, used as check. The varietal cross SINT4B with VS-536, both open pollinating synthetic maize varieties; Particulary, SINT 4B present short plant, semierect leaves, Good yield, White grain con and semident texture.

In reference to improved version of VS-536 it was focused in selecting short plant and ear, good plant and ear aspect, dent grain, good husk cover and tolerance to lodging; present nixtamal and flour quality and good acceptance by the industry (Sierra *et al.*, 2019).

Description of the experiment. During the spring summer season from 2016 to 2023, under rainy conditions, there was carried out an experiment, for evaluating 20 varietal crosses 5 experimental maize synthetics and three commercial genotypes, which of them, were distributed in complete blocks at random, with 28 treatments and three replications in plots of two rows 5 m long and 80 cm wide in a density of 62,500 plants ha^{-1} (Reyes, 1990). The fertilization was made according to the recommendations of INIFAP, Thus, in this Cotaxtla was utilized the formula 161-46-00, applying all the Phosphorus and a third part of Nitrogen at sowing moment, the rest of Nitrogen in bunchy stage using Urea as Nitrogen source; The weeds were controlled by Atrazine applied before emerging and there were controlled pests during developing crop.

Variables and data recording. During the development of the crop and at harvest time, there were recorded in the experiment the following agronomic variables: Grain yield, days to tassel and silking, Plant and ear height, qualification of plant and ear aspect and sanity, using a scale from 1 to 5, where, 1 correspond to the best phenotypic expression and 5 for the worst; lodging, ears with bad husk cover, total number of ears, dry matter and ear rot.

Statistical methods. The experimental design used was complete blocks at random with 28 entries and three replications in plots of two rows 5m long and 80 cm wide in a plant density of 62,500 pl ha^{-1}. Individual and combined analysis of variance was made for all variables recorded and were analyzed statistically and for the separation of means, the Significant Minimum Difference test was applied at 0.05 and 0.01 of probability (Reyes, 1990). On the other hand, comparisons of cross groups and synthetic parent varieties were made and the t-test at 0.05 and 0.01 probability was applied. Besides, the percentages of heterosis with respect to the best parent (Reyes, 1985), were calculated as follows:

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$$\% \text{ of Heterosis} = \frac{\text{F1} - \text{Best parent}}{\text{Best parent}} \text{ x } 100$$

Results and discussion

Advances in selection. Actually, there has been completed the four cicle of mass selection in the synthetic maize varieties, VS-536, the most used maize variety in the southeast of México. V-537C and SINT4B; The criteria of mass selection have been short plant and ear height for reducing lodging risk, Good husk cover, lodging tolerance, good plant and ear aspect and sanity, regular ears, white grain and dent texture in VS-536 and V-537C and semident texture in the SINT4B (Sierra *et al.*, 2019); The next agriculture season will start the characterization and the proceeding for official deliver of the new improved version of synthetic variety VS-536. This new versión of VS-536, present good plant and ear aspect and sanity; The relation ear height/plant height of 0.54, it is very important for lodging tolerance, present ears with 14 regular rows, good sanity white color and dent texture (Figures 1 and 2)

Figures 1 - 2 The new version of VS-536 present good plant and ear aspect and sanity with white grain and dent texture

Grain yield. From the combined análysis for grain yield in varietal hybrids across the 10 environments of evaluation, it was found statistical signifficance, at 0.01 of probability for Genotypes (G), for environments (E) and for interaction GxE. The signifficance for the interaction suggest that grain yield of the varietal hybrids across the environments is different (Reyes, 1990; Andrés *et al.*, 2017; Sierra *et al.*, 2018).

In this analysis, the mayor variance was registered for environments with value of 68.84**, it means that environments are different and important in the behaviour of varietal crosses; Besides, the coefficient of variation registered was of 13.44%, value relatively low, that suggest that the results of these experiments are reliables (Reyes, 1990). (Table 1)

Source of variation	DF	SS	MS
Genotypes (G)	27	92.07	3.41**
Environments (E)	9	394.78	68.84**
Interaction GxE	243	306.14	1.26**
Error	455		0.7151
CV (%)			13.44%

DF= Degree Free; SC= Sum of square MS= Mean Square; CV= Coefficient of variation; **= Signifficance for source of variation at 0.01

Table 1 Combined analysis of variance for grain yield of varietal maize hybrids across the 10 environments in Veracruz and Tabasco states. 2016 a 2023

The yield of varietal hybrids across the 10 environments identified the best ones at 0.01 of probability; These ones were: SINT2BxVS536, SINT4BxVS536, SINT4BxSINT2B, SINT5Bx VS-536, SINT5BxVS537C, SINT1BQxVS-536 and SINT3BxSINT1BQ, with grain yield from 6.39 to 6.96 t ha^{-1}, statistically similar to the commercial hybrid H-520, the most used in the southeast of México (Sierra *et al.*, 2019) Table 2

In the heterosis for the best varietal hybrids with respect to the best parent there were found values from 4.85 to 17.70%; The highest percentages of Heterosis were gotten for SINT2BxVS536 (17.70%), SINT4BxVS-536 (15.80%), SINT4B xSINT2B (10.78%), V537CxVS536 (11.21%).

Entry	Genealogy	Grain Yield[1/] t ha^{-1}	% Relative	% Heterosis
1	SINT2BxVS-536	6.96*	103	17.70
14	SINT4BxVS-536	6.84*	102	15.80
28	H-520	6.73*	100	
17	SINT4BxSINT2B	6.55*	97	10.78
16	SINT-5B x VS-536	6.48**	96	5.93
9	SINT-5B xV-537C	6.42**	95	4.91
13	SINT1BQxVS-536	6.41**	95	5.25
10	H-518	6.39**	95	
18	SINT3BxSINT1BQ	6.39**	95	4.85
12	SINT4BxSINT3B	6.37	95	7.75
19	SINT2BxVS537C	6.34	94	7.32
4	V-537CxVS-536	6.34	94	11.21
20	VS536xV537C	6.33	94	11.07
15	SINT5BxSINT1BQ	6.32	94	3.28
3	SINT5BxSINT4B	6.32	94	3.27
6	SINT3BxVS537C	6.31	94	7.24

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Entry	Genealogy	Grain Yield[1/] t ha^{-1}	% Relative	% Heterosis
11	SINT3BxSINT2B	6.30	94	6.54
2	SINT5BxSINT2B	6.29	93	2.81
25	SINT-5B	6.12	91	
5	SINT4BxVS537C	6.10	91	3.27
8	SINT4BxSINT1BQ	6.09	90	-0.03
21	SINT-1BQ	6.09	90	
7	SINT5BxSINT3B	6.07	90	-0.76
22	SINT-2B	5.91	88	
24	SINT-4B	5.91	88	
23	SINT-3B	5.88	87	
27	V-537 C	5.70	85	
26	VS-536	5.46	81	
	PROMEDIO	6.26		
	CV (%)	13.44		
	CME	0.7151		
	DMS 0.05	0.4279		
	DMS 0.01	0.5633		

[1/]= Mean Grain yield in 10 environments of evaluation; *and **/= Signifficance of genotypes at 0.05 and 0.01 of probability;

Table 2 Grain yield of varietal maize hybrids across 10 environments in Veracruz and Tabasco states 2016-2023

These Heterosis values suggests genetic divergence between the parental varieties (Reyes, 1985; Córdova *et al.*, 2007; Sierra *et al.*, 2004; Palemón *et al.*, 2012; Velasco *et al.*, 2019) (Figure 3).

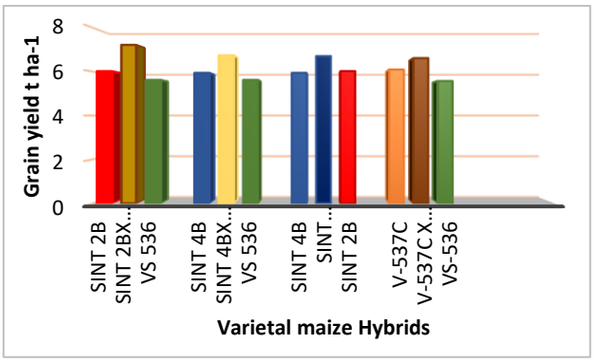

Figure 3 Heterosis in varietal maize hybrids Veracruz and Tabasco states 2016-2023

The best varietal hybrids were statistically similar to H-520, commercial hybrid used as check, and is the most used in the southeast of México; Besides these varietal hybrids take the advantage of maintaining only two parents, which are open pollinating maize varieties with greater yield, reliability, and easier for seed production (Sierra *et al.*, 2018; Sierra *et al.*, 2016; Gómez *et al.*, 2017; López *et al.*, 2021; Ramírez *et al.*, 2019; Tadeo *et al.*, 2021). In the best varietal hybrids participate VS-536, the synthetic maize variety of greater use in the Mexican southeast (Sierra et al., 2016).

Instead of grain yield and agronomic traits, it is suggested that the varietal cross SINT4BxVS-536, can be registered as new maize hybrid for the humid tropic in México. This varietal hybrid, present good yield, adaptation to the humid tropic of México, short plant and ear, good plant and ear aspect and sanity and good husk cover (Figures 4 and 5).

Environmental indexes. Instead of environmental indexes, Carlos A. Carrillo, Ver. 2016B and Cotaxtla, Ver., 2016B, recorded the highest yield with 7.29** and 7.27** t ha^{-1} and the greatest environmental indexes, 1.03** and 1.01** for each environment, respectively.

Figures 4 - 5 SINT4BxVS-536 present short plant and ear, good plant and ear aspect and, Good husk cover, white grain and semident texture

On the other hand, the locations Huimanguillo Tabasco in 2018 and 2016B registered the lowest grain yield with 5.40 and 5.18 t ha^{-1}, and negative environmental indexes of -0.86 and -1.08, (Table 3). It suggest, that there are important differences in these environments in clime, soil and agronomic management for these experiments (Reyes, *et al.*, 1990; Sierra *et al.*, 2018).

Environment	Grain yield t ha^{-1}	Env indexes
Carlos A. Carrillo, 2016B	7.29**	1.03
Cotaxtla 2016B	7.27**	1.01
Cotaxtla 2018B	6.48	0.22
Carlos A. Carrillo, 2023A	6.46	0.20
Huimanguillo 2023A	6.39	0.13
Cotaxtla 2022B	6.36	0.10
Cotaxtla 2017B	6.03	-0.23
Cotaxtla 2023A	5.72	-0.54
Huimanguillo 2018B	5.40	-0.86
Huimanguillo, Tab 2016B	5.18	-1.08
Mean	6.26	

Table 3 Environmental indexes in varietal maize hybrids 2016-2023. CIRGOC INIFAP

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Comparisons and t test. From the comparisons and t test at 0.05 and 0.01 of probability, there was found that the varietal hybrids recorded an average grain yield of 6.38 t ha^{-1} , 9 % more than the synthetic varieties parents with value for the calculated t test of 5.07**; Besides, there was registered advantages in plant and ear aspect (Reyes, 1990). It suggests that there is genetic divergence between the parents, which is also reflected in the values of heterosis with respect to the best progenitor that varied from 4.85 to 17.70 % (Reyes, 1985; Sierra *et al.*, 2004; Córdova *et al.*, 2007; Palemón *et al.*, 2012; Velasco *et al.*, 2019) (Table 4).

Comparison	Grain yield t ha^{-1}	% Rel	t Calc	Plant heigh	% Rel	t Calc	Plant Asp2/	% Rel	t Calc	Ear Asp2/	% Rel	t Calc
Crosses	6.38	109	5.07**	231.75	103	0.93NS	2.25	100	0.92NS	2.43	100	0.57NS
Parents	5.87	100		225.57	100		2.37	105		2.51	103	

t0.05 (54 GL) = 2.00; t0.01 (54 GL) =2.66. % Rel= Relative percentage; t Calc= t calculated; plant asp= plant aspect; ear asp = Ear aspect; 2/= Qualification scale from 1 to 5, where, 1 correspond to plants and ears with the best phenotypic expression and 5 for the worst

Table 4 Comparisons and t test for varietal maize hybrids and their parents. CIRGOC 2016-2023

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Conclusions

The outstanding varietal hybrids at 0.05 probability were: SINT-2BxVS-536, SINT-4BxVS-536, SINT-4BxSINT-2B, with grain yield from 6.55 to 6.96 t ha^{-1}, yield statistically similar to that recorded by the commercial hybrid H-520.

Heterosis percentages with respect to the best parent in the outstanding varietal crosses were: SINT-2BxVS-536 (17.70%), SINT-4BxVS-536 (15.80%), SINT-4BxSINT-2B (10.78%), V-537CxVS-536 (11.21%).

The crosses recorded an average yield of 6.38 t ha^{-1}, 9% more in relation to the parents, as well as better plant and ear aspect scores.

Based on grain yield and agronomic characteristics, the cross SINT-4BxVS-536 is proposed for official registration with SNICS as HV-570, New maize hybrid for the humid tropics of Mexico.

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Antibacterial activity of orégano *(Lippia graveolens)* essential oil against *Staphylococcus aureus in vitro*

Actividad antibacteriana de aceite esencial de orégano (*Lippia graveolens*) contra *Staphylococcus aureus in vitro*

VARGAS-MONTER, Jorge†´, SIFUENTES-SAUCEDO, Diana María*´, ZARAGOZA-BASTIDA, Adrián´´ and NIETO-AQUINO, Rafael´´´

´ *Universidad Politécnica de Francisco I. Madero. Tepatepec, Francisco I Madero, Hgo., CP. 42660. México*.

´´*Área Académica de Medicina Veterinaria y Zootecnia, Instituto de Ciencias Agropecuarias, Universidad Autónoma del Estado de Hidalgo, Tulancingo, México*.

´´´ *Tecnologico Nacional de México. Campus Ciudad Valles. Carr. al Ingenio Plan de Ayala Km.2, Col. Vista Hermosa. Cd. Valles, S.L.P. C.P. 79010. México.*

ID 1st Author: *Jorge, Vargas-Monter* / **ORC ID:** 0000-0001-8384-7941

ID 1st Co-author: *Diana Maria, Sifuentes-Saucedo* / **ORC ID:** 0000-0001-8384-7941

ID 2nd Co-author: *Adrian, Zaragoza-Bastida* / **ORC ID:** 0000-0002-8537-5025

ID 3rd Co-author: *Rafael, Nieto-Aquino* / **ORC ID:** 0000-0003-1661-8043

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Abstract

Mastitis, an inflammatory condition of the mammary gland, prevalent in dairy cows, affects production, milk sanitary quality and production costs. Therefore, the objective of this study was to evaluate the antibacterial activity of oregano essential oil (*Lippia graveolens*) against *Staphylococcus aureus* asosida in bovine mastitis, under in vitro conditions. Mastitis-positive milk samples were collected and subjected to the California field test (MCT). Isolation and biochemical identification of Staphylococcus aureus strains associated with mastitis was performed. The extraction of oil from Lippia graveolens leaves was carried out by Soxhlet extraction method. The antimicrobial test of oregano oil was determined by the disk diffusion method, 5, 10 and 25 µl of oil were evaluated at times of 24 and 48 h, distilled water was used as a negative control and chloramphenicol as a positive control. The antimicrobial activity showed significant differences, the concentration of 25 µl of oil presented the best antimicrobial activity, compared to the negative control, which showed the highest bacterial growth. There were no significant differences at 24 and 48 hours. The use of *Lippia graveolens* oil has antibacterial activity against *Staphyloccus aureus*, determined by the concentration of the oil and with no difference in the effect of the exposure time.

Bovine mastitis, Bacteria, Plant extract

Resumen

La mastitis, condición inflamatoria de la glándula mamaria, prevaleciente en las vacas lecheras, afecta la producción, la calidad sanitaria de la leche y costos de producción. Por tanto, el objetivo del trabajo fue evaluar la actividad antibacteriana de aceite esencial de orégano (*Lippia graveolens*) contra *Staphylococcus aureus* asociada a mastitis bovina, bajo condiciones in vitro. Se colecto muestras de leche positivas a mastititis, a prueba de campo test de california (MCT). Se realizo aislamiento e identificación bioquímica de cepas de *Staphylococcus aureus* asociadas a mastitis. La extracción del aceite de las hojas de *Lippia graveolens*, se efectuó por método de extracción Soxhlet. La prueba antimicrobiana del aceite de orégano se determinó por el método difusión en disco, se evaluó 5, 10 y 25 µl de aceite en tiempos de 24 y 48 h, se uso agua destilada como control negativo y el cloranfenicol, como control positivo. La actividad antimicrobiana mostro diferencias significativas, la concentración de 25µl de aceite presento la mejor actividad antimicrobiana, comparado con el testigo negativo el cual observo el mayor crecimiento de la bacteria. No existe diferencias significativas a las 24 y 48 h. El uso de aceite de *Lippia graveolens* tiene actividad antibacteriana contra *Staphyloccus aureus*, determinada por la concentración del aceite y sin diferencia en el efecto por el tiempo de exposición.

Mastitis bovina, Bacterias, Extracto vegetal

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* Author's Correspondence (E-mail: dmsifuentes@upfim.edu.mx)

† Researcher contributing as first author.

Introduction

Mastitis is a pathology of the mammary gland, with an important prevalence in dairy cows, affecting milk production and sanitary quality and increasing production costs due to the use of antibiotics in the treatment of sick animals (Vargas *et al*., 2021). There are more than 100 microorganisms causing intramammary infections in cattle, with bacteria being the most frequent (Hogan & Smith, 2001). Mastitis-causing bacteria have been classified by Gram staining into positive and negative, contagious and environmental according to their means of transmission (Lakew *et al*., 2019 and Cheng et al., 2020). Contagious bacteria such as *Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplama bovis* and *Corynebacterium*, are located in the udder and teat skin of the cow, and can colonize the teat canal, so in poor routine milking processes they are transmitted from cow to cow giving rise to subclinical mastitis, with high somatic cell counts and reduced milk production (Silva, 2021).

Staphylococcus aureus, a Gram-positive contagious bacterium with a low degree of infection, causes chronic mastitis and loss of milk secreting tissue (Cheng *et al*., 2020). It can produce capsular polysaccharides (CPs) that confer resistance against phagocytosis by polymorphonuclear neutrophils in cattle, some strains are able to produce biofilm, cell aggregates surrounded by an extracellular matrix and are a significant virulence factor in the persistence of infections in case of subclinical and chronic mastitis (Gomes *et al*., 2016; Schönbor *et al*., 2017 and Toledo *et al*., 2021). Biofilms influence the susceptibility of microbial agents, which is why several strains of *Staphylococcus aureus* currently exhibit antimicrobial resistance. Bacterial multiresistance to antimicrobials may be herd-specific and influenced by management and environmental conditions (Pascu *et al*., 2022 and Widianingrum *et al*., 2022).

The WHO (2020) mentions that drug-resistant bacteria can cause infections that are difficult to treat, increasing mortality. In view of this problem, new therapeutic alternatives are being sought to combat bacterial infections through the use of medicinal plant extracts.

The antibacterial effect of extracts has been evaluated in disc diffusion bioassays, wells, agar broth and agar dilution methods (Punniamurthy *et al*., 2017 and Liu *et al*., 2017). Bioactive phytochemical compounds in plants such as alkaloids, flavonoids and saponins are responsible for their antimicrobial potential (Kebede *et al*., 2021 and Amber *et al*., 2018).

The selection of plants for phytochemical studies and bioassays directed against bacteria is done in terms of plant availability, knowledge of medicinal properties by the population and low cost, with the aim of being an option in local livestock health care (Amber *et al*., 2018). Oregano is a plant that grows wild in 24 states of Mexico (De la Cruz *et al*., 2007) and has been studied for its antimicrobial bioactive compounds such as carvacrol and thymol (Kovačević *et al*., 2021). The antimicrobial activity depends on the chemical composition of oregano essential oil, which is related to the oregano species, geographical conditions, harvesting periods and extraction method (Bautista *et al*., 2021 and Aires *et al*., 2016). The aim of this work is to evaluate the antibacterial activity of wild oregano (*Lippia graveolens*) extract against Staphylococcus aureus under in vitro conditions.

Materials and methods

The study was carried out in the animal phytotherapy experimental laboratory of the Universidad Politécnica Francisco I. Madero, in the municipality of Francisco I. Madero in the state of Hidalgo. The site is located at an altitude of 1 995 m above sea level, with geographical coordinates of 20° 15' 20" north latitude and 99° 00' 10" west longitude. It has a cold temperate climate, with a mean annual temperature of 17 °C and an annual rainfall of 540 mm.

Plant material

The plant material of *Lippia graveolens* Kunth was collected in the spring-summer period of 2022, in the town of Orizabita in the municipality of Ixmiquilpan. For the taxonomic identification of the plant, the herbarium of the Universidad Nacional Autónoma de México (UNAM) was consulted and the correspondence of *Lippia graveolens* Kunth (IBUNAM:MEXU:308815) was verified.

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Obtaining the oil

The leaves of *Lippia graveolens* Kunth were dried in the shade at room temperature for 72 h and placed for 24 h in a conventional oven at 45°C to completely remove moisture (López *et al*., 2022). The extraction of oil from oregano leaves was carried out by Soxhlet extraction method, 60 grams of ground sample per 230 mL of petroleum ether. After the distillation cycles were completed, the remaining liquid was passed to the rotary evaporator to extract the solvent and vacuum filtration was performed to remove the residues of the plant material. The extract was collected in amber glass bottles to avoid degradation by light and stored at 4°C until use.

Collection of milk samples

Milk samples were collected from cows in the dairy cattle production module of the university. The California Mastitis Test (alkyl-aryl sodium sulfonate) (masitest®) was performed using the CMT paddle, 2 ml of milk was taken from each udder quarter (Philpot and Nickerson, 2000). and placed in each well of the paddle to be mixed homogeneously with 2 ml of reagent for 20 seconds. The reaction to the test was interpreted on a graded ordinal scale for subclinical mastitis: Negative (0), Trace (T), 1, 2, and 3, these values being correlationally indicative of the presence of somatic cells. Prior to sampling, the mammary gland was aseptically sterilised to avoid contamination at the time of sampling, and the tip of the teat was disinfected with a swab soaked in 70% alcohol. The first stream of milk was removed from the teat and samples of 50 ml of milk were collected in sterile bottles.

Isolation and identification of Staphylococcus aureus

106 dilutions of 1mL of milk were made for seeding in petri dishes with two selective culture media: mannitol salt agar and blood agar supplemented with colistin and nalidixic acid. The boxes were incubated for 48 hours at 37°C for macroscopic evaluation. Biochemical tests such as gram stain, oxidase and catalase were performed for bacterial confirmation.

Antibacterial activity tests

For the antimicrobial test analysis of oregano oil was determined by the disc diffusion method (disc-placculture method) (Albado *et al*. 2001). Diluted strains were mass seeded on blood agar, four 7 mm diameter filter paper discs (Whatman) impregnated with 5, 10 and 25 µl of oil were placed on the surface of the media. Distilled water was used as a negative control and chloramphenicol (30 µg/ml) as a positive control, incubated at 37°C for 24 and 48 hours. Both tests were performed in triplicate. The diameter of the growth inhibition halo of the microorganisms was measured and the calculation of the percentage of the relative inhibitory effect with respect to the positive control was estimated by applying the expression (Corzo *et al*., 2012):

%Inhibitory effect=(Inhibition halo diameter of the extract-Inhibition halo diameter of the negative control)/(Inhibition halo diameter of the positive control-Inhibition halo diameter of the negative control) X 100.

Statistical analysis

An analysis of variance was applied to determine the effect of treatment on bacterial inhibition. For the comparison of means between treatments, Tukey's test was performed. Significance was reported at a 95% confidence level ($p \leq 0.05$). SAS software, version 9.0 (SAS, 2006) was used.

Results and discussion

Bacterial identification by chemical tests showed Gram-positive, catalase-positive and oxidase-negative bacteria. On selective culture media for identification of morphology on Mannitol Agar medium, macroscopically smooth, raised, shiny, full-bordered colonies with creamy consistency and golden yellow pigmentation characteristic of Staphylococcus aureus were found. On blood agar medium, the presence of Staphylococcus aureus bacteria was evidenced by the production of β-hemolysis generating a clear zone around the colony as referred to by Cervantes *et al*.

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Significant effect ($p \leq 0.05$) per treatment and significant differences between treatments were found according to the tukey test ($p \leq 0.05$) with the exception of the 25µl concentration treatment, there is no significant difference between 24 and 48 hours (Table 1). The 5µl essential oil concentrations showed on average a lower halo and inhibition effect on S. aureus. The 25µl concentration of oil showed the best antimicrobial activity. The highest bacterial growth was observed in the negative control control control.

Oil concentration	Inhibition Halo (mm)		Relative effect of Inhibition (%)	
	24	48	24	48
5µl	7.07^{c}	6.13^{c}	40.8^{c}	35.9^{c}
10µl	8.8^{b}	8.53^{b}	56.9^{b}	55.4^{b}
25µl	12.27^{a*}	12.0^{a}	82.2^{a}	83.7^{a}
Biodistilled water	1.93^{d}	1.73^{d}	-	-
Chloramphenicol (30 mg)	14.5^{a}	14.0^{a}	-	-
* Different letters indicate significant statistical difference (Tukey, P≤0.05)				

Table 1 Antimicrobial activity of essential oil of *Lippia graveolens* against a field strain of *Staphylococcus aureus*

The best in vitro test result was achieved with the 25 µl oil concentration at 24 and 48 hours where the highest percentage of relative inhibition against Staphylococcus aureus was obtained. An antibacterial action is considered high when its relative inhibition percentage is greater than 70%, intermediate between 50-70% and low when it is less than 50% (Ramirez and Diaz, 2007). Consequently, the 25 µl concentration has a high antibacterial action and the 10 µl concentration has a high intermediate activity at 24 and 48 hours. In relation to the positive and negative control, the values indicate that chloramphenicol was superior to the treatments, however, the antimicrobial activity of oregano oil is significant and similar to that reported by Gallegos et al., (2019) in a study of biological activity where they found an inhibition halo effect of 11.5 mm at a concentration of 20 µL for Staphylococcus aureus, similar to their positive control Cephalexin (36 mg/mL).

The antibacterial activity of Mexican oregano oil has been reported against gram-positive bacteria such as S. aureus and gram-negative bacteria such as E. coli, A. baumanii and Pseudomona sp (Delgadillo et. al, 2017 and Carhuallanqui et al., 2020). It is their phytochemical compounds such as thymol and carvacrol, monoterpenes with a phenolic ring consisting of two isoprene molecules with three functional group substituents (Memar et al., 2017), that have antibacterial, antioxidant, anticancer and anti-inflammatory properties (Sharifit et al., 2021). These compounds alter the proteins and lipids of the bacterial cell wall and cytoplasmic membrane, affect RNA synthesis and ATPase activity and induce an imbalance in intracellular osmotic pressure (Tapia-Rodriguez et al., 2017).

Conclusions

The present study demonstrated that Lippia graveolens oil has antibacterial activity against Staphylococcus aureus, determined by oil concentration and with no difference in effect by exposure time. The antimicrobial activity is associated with the phytochemical composition of the plant and the susceptibility of the bacteria. Further research on the phytochemical composition of the plant and the factors affecting it is suggested to determine its potential for use in phytotherapy against bacteria causing bovine mastitis.

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Response of cotton (*Gossypium hirsutum* L.) seeding the ultra-narrow grooves and high population density

Respuesta del algodón (*Gossypium hirsutum* L.) a la siembra en surcos ultra-estrechos y altas densidades poblacionales

MARTINEZ-LARA, Filiberto*†, CHINO-CANTOR, Araceli, MARTÍNEZ-SÁNCHEZ, Itzcóatl and CIPRIANO-ANASTASIO, Juan

Unidad Académica Metztitlán, Universidad Politécnica de Francisco I. Madero. Av. Tepeyacapa S/N, Metztitlán, Hidalgo, C.P. 43350, México

ID 1st Author: *Filiberto, Martínez-Lara* / **ORC ID:** 0009-0004-4887-920X, **CVU CONHCYT ID:** 371869

ID 1st Co-author: *Araceli, Chino-Cantor* / **ORC ID:** 0009-0006-7925-4506, **CVU CONHCYT ID:** 419009

ID 2nd Co-author: *Itzcóatl, Martínez-Sánchez* / **ORC ID:** 0000-0002-3114-319X, **SNI CONHCYT ID:** 311256

ID 3rd Co-author: *Juan, Cipriano-Anastasio* / **ORC ID:** 0000-0001-7740-6453, **CVU CONHCYT ID:** 422557

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Abstract

The cotton planting (*Gossypium hirsutum* L.) on narrow rows instead on conventional rows to 75 cm, show to be an alternative to increasing crop yield and to reduce production costs. The objective of this research was to study the effects of ultra-narrow rows and plant density on the biomass production and fiber quality. Three-row spacings and three plant densities were evaluated in the Comarca Lagunera, México. Ultra-narrow rows 50 and 35 cm, apart and conventional rows spaced to 75 cm, were used. The population densities were 80,000, 100,000 and 120,000 plants ha-1. The conventional variety Fiber max 98 was used. The nine treatments were assigned to a randomized complete block design with three replications in a split plot arrangement. Row spacings were assigned to main plots and the population densities to subplots. Seed-cotton and lint yield were evaluated in kg ha^{-1}, yield components (boll weight, lint percentage and seed index), fiber quality (length, resistance and fineness) and the plant growth indexes, leaf area index (LAI), net assimilation rate (NAR), crop growth rate (CGR), specific leaf area (SLA), leaf weight fraction (LWF) and leaf area ratio (LAR). Seed-cotton yield was different ($P<0.01$) to 75, 50 and 35 cm, row spacings, with 4,504, 5,377 and 6,259 (Kg ha^{-1}), respectively. The highest yield was obtained in 35 cm, row spacing, which was higher 15 and 29% to the obtained on 50 and 75 cm, row spacings, respectively. The fiber quality was not affected by row spacings and plant density. Row spacing did not affected the plant growth indexes measured. The cotton production system on 35 cm rows with a plant density of 120,000 plants ha^{-1} can be an alternative to increasing yield and to reduce production costs, without yield reduction. Getting more profit for the producer.

Fiber quality, Cotton yield, Growth rates

Resumen

La siembra de algodón (*Gossypium hirsutum* L.) en surcos más estrechos que los convencionales a 75 cm o más, sugiere ser una alternativa para aumentar el rendimiento y reducir los costos de producción. Con el objeto de conocer el efecto que los surcos ultra-estrechos y la densidad poblacional tienen sobre el potencial productivo, de biomasa y calidad de fibra, se estudiaron tres distancias entre surcos en la Comarca Lagunera, México. Se utilizaron las distancias a 50 y 35 cm como surcos ultra estrechos comparados con el testigo a 75 cm, combinados con tres densidades de población a 80, 100 y 120 mil plantas ha^{-1}, con la variedad convencional Fiber max 98. Los nueve tratamientos se distribuyeron al azar en un arreglo de parcelas divididas y tres repeticiones. Las distancias entre surcos se asignaron a la parcela mayor y, las densidades de población a la parcela menor. Se evaluó el rendimiento de algodón en hueso y pluma (fibra) en kg ha^{-1}, componentes de rendimiento (peso de capullo, porcentaje de pluma e índice de semilla), calidad de fibra (longitud, resistencia y finura) y seis índices relacionados con el crecimiento. El rendimiento en hueso fue significativamente diferente ($P<0.05$) para los distanciamientos 75, 50 y 35 cm con 4,504, 5,377 y 6,259 (Kg ha^{-1}) respectivamente. La superioridad a 0.35 cm en rendimiento se relacionó con una mayor densidad poblacional. La calidad de fibra no fue afectada por la distancia entre surcos ni por la densidad poblacional. El sistema de producción a 35 cm es una alternativa para incrementar los rendimientos y reducir costos de producción.

Calidad de fibra, Rendimiento de algodón, Índices de crecimiento

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* Author Correspondence (E-mail: malfi1982@gmail.com)
† Researcher contributing as first author.

Introduction

A traditional and recurrent problem for cotton producers is the reduced profitability of their crop, due to the constant increases in production costs and the low price of the fibre on the international market, since the price of the fibre is subject to production, reserves and world demand for it. In view of this situation, new alternatives have been explored to increase unit yields and make the crop more profitable.

Increasing unit productivity and reducing costs require genotypes with greater photosynthetic efficiency and new production systems. Work is currently underway to induce morphological changes (number of nodes, plant height) and physiological changes (earliness, synchronisation between vegetative and reproductive weight ratio) to increase the efficiency of fibre production. This is sought through the adaptation or modification of cultivation practices and the reduction in the application of inputs, as long as productivity is not affected.

As a result of this research, the ultra-narrow furrow cotton production system with high stocking densities was developed. The concept of ultra-narrow furrows (furrows less than 75 cm apart) dates back to 1920, Perkins et al. (1998). The objective at that time, as it is today, is to reduce production costs. Lewis (1971) concluded that the reduction of production costs with the ultra-narrow furrow system could be derived from the shortening of the crop cycle.

Because the cotton plant fruits in an orderly and sequential manner, emitting a flower at regular 3-day intervals on successive fruiting branches and at 7-day intervals between flowers on the same fruiting branch, with the ultra-narrow furrow production system and increased stocking density, fewer fruits per plant would be required to maintain current yields.

Therefore, if fewer acorns are needed to maintain these yields, the time required to obtain them would be less than in the conventional planting system (furrows spaced 75 to 100 cm apart).

Lewis (1971) pointed out that in the ultra-narrow furrow production system, plants could exhibit their fruiting structures at very identical stages of development throughout the cycle. This growth characteristic contrasts with that of the conventional planting system which exhibits fruiting at widely varying stages of development during the flowering and acorn ripening period. A more synchronised flowering pattern would lead to more efficient chemical pest control, and the regulation of plant growth with phyto-regulators would increase the possibility of increasing unit production.

Allen (1998) points out that shortening the crop cycle would lead to a reduction in the number of insecticide applications to protect fruitlets. Reduced row spacing and increased stocking density induces earlier crop closure than in conventional rows George, (1971). Faster ground cover reduces the critical period of weed competition Snipes, (1996), increases solar radiation interception and decreases evaporative water loss Kreig, (1996).

In West Texas, it was determined that in the conventional planting system (90 to 100 cm furrows), 40% of the water available to the crop is lost by evaporation, so the use of ultra-narrow furrows would allow more water to be absorbed by the plant, instead of being lost by evaporation. Gerik *et al.* (1998) report that sowing in ultra-narrow furrows increases yield by up to 37 % and reduces the crop cycle by 12 days compared to sowing in 76 cm furrows. While Cawley et al. (2002) report more modest yield increases (5 to 11 %) with a 7 to 10 days reduction in the crop cycle compared to planting in 0.92 m furrows. Prince et al. (2002) point out that with this technology it is possible to increase unit yields, reduce the crop cycle, control excessive plant growth, reduce production costs, etc. Gaytán et al. (2004) found no differences in yield when sowing in furrows spaced at 50 and 76 cm, nor between population densities that ranged between 80 000 and 200 000 plants ha-1, but indicated that sowing in 50 cm furrows reduces the crop cycle by seven days. Earliness and/or shortening of the crop cycle is a characteristic that confers resistance (pseudo-resistance) to pest damage by allowing the plants to escape damage from later generations of damaging insects.

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Another quality of earliness, and the shortening of the crop cycle, is that of escaping adverse environmental conditions such as low temperatures or rainy periods that can affect yield and fibre quality. However, some researchers indicate that fibre quality can be affected by moisture or N deficiencies. Mark et al. (2002), or by differences between varieties, stocking density, row spacing, year effect, or any of their interactions. Mohamad et al. (1982).

Methodology

The research was carried out at the Experimental Field of the Universidad Autónoma Agraria Antonio Narro, Laguna Unit, in Torreón, Coah. It is located between parallels 25° 42' and 24° 48' north latitude and meridians 103° 31' and 102° 58' west longitude at an altitude between 1 000 and 2 500 m. INEGI (2009). Three furrow spacings were studied; 0.75 (control), 0.50 and 0.35 m (ultra narrow furrows) and three stocking densities, 80,000, 100,000 and 120,000 plants ha^{-1}.

The variety used was Fiber max 989, the treatments were distributed in a split plot arrangement, locating the distances between rows in the large plot and the stocking densities in the smaller plot. The large plot design was a randomised block design with three replications. The large plot consisted of 8 furrows of 5 m in length and the useful plot, for yield and biomass production and distribution data, consisted of 6 furrows of 4 m in length. Planting was carried out on dry soil. Fertilization was done with the formula 100-30-00 (N-P-K), then one sowing irrigation and three auxiliary irrigations were applied at 73, 93 and 108 dds.

During the cycle, the problem pests were the armyworm (Spodoptera exigua), which was controlled by applying a mixture of cypermethrin at a rate of 0.5 L ha-1 together with chlorpyrifos at a dose of 1.5 L ha-1 and the silverleaf whitefly (*Bemisia argentifolii*), for which Endosulfan was applied at a dose of 2.3 L ha-1. The weeds were controlled manually. Crop closure was estimated from measurements of horizontal plant growth, in cm. Crop closure was considered when the branches of the plants were joined together. The earliness at the beginning of flowering was evaluated in days after sowing (DDS). From the appearance of the first flower buds, the growth dynamics in height were recorded weekly.

The cotton yield in stone (RAH) and feather (RAP) in kg ha^{-1} was estimated. For this variable, two 6 m long rows per plot were harvested manually. In a sample of 20 cocoons taken at random per plot, the following yield components were evaluated: cocoon weight (CW), fibre percentage (FP), and seed index (SI), which is the result of the weight of 100 seeds. The sample of 20 cocoons was de-seeded by separating the fibre from the seed, which was used to determine the percentage by weight of the 20 cocoons. For the determination of fibre quality, the dehulled sample of the 20 cocoons harvested per plot was sent to the fibre laboratory of CIRNOC INIFAP, where they were analysed to obtain the values for fineness, fineness and quality (MIC) by means of micronaire, fibre length (LEN) in mm and fibre strength (STR) in (KNm kg^{-1}).

The dynamics of dry matter production and its allocation were measured in three destructive samplings at 74, 94 and 136 dds. In each sampling, two plants per plot were taken in full competition and divided into four subsamples; stem, branches, leaves and fruitlets. Each subsample was placed in a separate paper bag. The subsamples were taken to dry weight for which they were placed in a drying oven at a temperature of 65oC for a period of 72 hours. They were then weighed to obtain the dry weight. The sum of the weights of stem, branches and leaves indicated the amount of biomass accumulated in the vegetative organs. The sum of the dry weights of vegetative and fruiting organs gave the total dry weight per plant. To obtain the leaf area per plant, the area of subsamples of leaf laminae was measured in groups of different sizes and the dry weight of each group was also determined. With the information obtained, a simple regression analysis was carried out in which the dependent variable (Y) was the leaf area and the independent variable (X) was the dry weight of the subsamples.

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With the dry matter values, the following growth rates were calculated, according to Radford (1967 and Hunt (1978). Crop growth rate (CGR), measures the increase in biomass per unit time, Net assimilation rate (NAR), estimates the photosynthetic efficiency of the plant, Leaf Area Ratio (FAR), is an indicator of the size of the photosynthetic apparatus of the plant, and is obtained by dividing the leaf area of the plant by the total dry weight of the plant, Specific Leaf Area (SFA), measures leaf thickness and represents the leaf area per gram of leaf dry weight, Leaf Weight Ratio (LWR), determines the distribution of assimilates to the leaves, and is an indicator of plant leafiness and Leaf Area Index (LAI), is the leaf area per unit of soil surface area, generally 1 m^2. The data were analysed with the SAS statistical programme, using the combined analysis of variance procedure including distance between rows and population density. The DMS test ($P \leq 0.05$) was used for the comparison of means.

Results

Yield, yield components and growth indices

The analysis of variance showed significant effect ($P<0.05$) for yield which was not the case for the other factor interactions.

Row spacing showed significant difference ($p<0.01$) in boll cotton yield (RAH) and feather cotton yield (RAP); for boll weight (BW), % fibre (FP), seed index (SI) and plant height (PA), it was not statistically significant (Table 1). These results coincide with those reported by Palomo, (2007), who found significantly higher yields of seed cotton, feather cotton and cocoons per m^2 for the 35 cm row spacing compared to the 50 and 75 cm spacings. Estrada, (2008), in consecutive years found higher and statistically different yields ($P \leq 0.05$) when sown in 35 cm furrows than in 50 and 75 cm furrows, whose average yield at 35 cm spacing was 10 % higher than at 50 cm and 26 % higher than at 75 cm (control). Vories and Glover, (2006), found higher yields for cotton planted at 19 cm compared to 97 cm rows, where the cocoons per m2 component determined the yield advantages of seed cotton and seed cotton of 35 cm rows over 50 and 75 cm rows. Palomo et al. (2007), concluded that ultra-narrow furrows yield 16% more seed cotton than planting in 75 cm furrows.

The boll weight and seed size tend to decrease as the row spacing is reduced and they claim that the transgenic and conventional varieties have the same potential.

Groove distances (cm)	Performance (Kg ha^{-1})		Cocoon Weight (g)	% of fibre	Seed rate	Plant height (cm)
	Bone	Pen				
75	4504.1 c	1967 c	5.8 a	43.8 a	9.1 a	76.4 a
50	5377.0 b	2396.1 b	6.0 a	44.5 a	9.1 a	77.4 a
35	6259.1 a	2733.1 a	5.9 a	43.6 a	8.0 a	70.5 a
Media	5380	2365	5.9	43.9	9	74.7

Table 1 Yield (kg ha^{-1}) and components at three row spacings
Similar letters are statistically equal DMS ($P \leq 0.05$).

The population density did not show significant difference between boll cotton yield (BHY) and seed cotton yield (YYP), nor for cocoon weight (CW), % fibre (FP), seed index (SI) and plant height (PA). However, it is observed that the density of 100,000 plants ha^{-1} shows the highest values (Table 2).

Palomo et al. (2007) found that planting in 35 cm furrows at a stocking density of 98,000 plants ha^{-1} showed the highest yields, which yielded 22 % more than planting in 50 cm furrows at 80,000 plants ha^{-1} and 27 % more than planting in 75 cm furrows at 67,000 plants ha-1, with the exception of bud weight, the other yield components were not affected by the production system. Gaytán *et al*. (2004) found no statistically significant differences for the values of yield components, boll weight and seed index and also confirmed the absence of response for cotton yield at the different row spacings and stocking densities evaluated. Palomo et al. (2007) found that population densities do not affect yield and its components in their study only there was difference in seed index which decreased with increasing population density and conclude that reduction of row spacing and in conjunction with increase in population density increase biomass production and yield of cotton.

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Plants (m^2)	Performance (Kg ha^{-1}) Pen Bone	Cocoon Weight (g)	% of fibre	Seed rate	Plant height (cm)
8	5391.4 a 2361.4 a	6.0a	43.9a	9.1a	75.7a
10	2403.9 a 5493 a	5.9a	43.6a	8.9a	72.0a
12	5255 a 2331.4 a	5.9 a	44.3 a	9.1a	76.6a
Media	5379 2,365	5.9	43.9	9.0	74.7
Similar letters are statistically equal DMS (P≤ 0.05).					

Table 2 Yield (kg ha^{-1}) and components at three stocking densities

Fibre quality only showed a significant difference in the fineness component, where the 50 and 35 cm spacings were better than the 75 cm spacing, with a better trend observed in the 50 cm spacing. While in stocking density, a significant difference was observed for fibre strength where the 75 and 50 cm spacings were better than the 35 cm spacing. Palomo et al. (2001) mention that generally in years with high temperatures, fibre with lower length and strength but greater thickness is obtained. The results found by Chavarría (1998), coincide with the results obtained where fibre strength had a tendency to decrease as plant density increased.

Variation factors Row spacing (cm)	Length (mm)	Resistance (KNm kg^{-1})	Fineness (Micronaire)
75	1126.0 a	27.0 a	4.3 b
50	1121.0 a	27.2 a	4.5 a
35	1134.2 a	27.1 a	4.4 a
Average	1127	27.1	4.4
Stocking density			
Plants (m^{-2})	1127.3 a	27.0 a	4.4 a
10	1135.4 a	27.5 a	4.4 a
12	1118.4 a	26.7 b	4.4 a
Media	1127	27	4.4
Similar letters are statistically equal DMS (P≤ 0.05)			

Table 3 Average cotton fibre quality variables for the main sources of variation at three row spacings and three stocking densities.

The analysis of variance for all growth indices only showed a significant difference in the leaf weight ratio (LWR), which determines the distribution of assimilates to the leaves and is an indicator of the leafiness of the plant. The distance of 75 cm was the best to those of 50 and 35 cm, (table 5). The other indices did not show any difference.

It is necessary to comment that from the first sampling (74 dds) to (136 dds), the 35 cm distance showed a tendency to be better by obtaining higher crop growth rate (CGR) and net assimilation rate (NAR) than the other indices (table 4). It is observed that the highest values of leaf area ratio (LAR) and leaf weight ratio (LWR) occurred in the early stages of plant growth, and that they tend to decline with increasing crop age. This is due to the fact that in the early stages of growth, plants invest most of the photoassimilates in the establishment of their photosynthetic apparatus, an amount that gradually decreases as the plant accumulates more carbohydrates in other plant organs, especially in the reproductive organs. Palomo et al. (2003).

INDEXES	plants (m^{-2})	SAMPLING 1º 0-74	2º DDS 74-94	3º 94-136
TCC (g m^{-2} day^{-1})	8	24.3a	15.9a	10.5a
	10	25.2a	18.5a	12.5a
	12	26.9a	15.4a	10.4a
TAN (g ms m^{-2} day^{-1})	8	12.8a	12.7a	11a
	10	12.6a	13.4a	12.1a
	12	14.5 a	13a	12.7a
IAF (cm^2 g^{-1})	8	1.1a	2.4a	2a
	10	1.1a	2.5a	1.9a
	12	1 a	2.5a	1.7a
Similar letters are statistically equal DMS (P≤ 0.05).				

Table 4 Growth rates of conventional Fiber Max 989 cotton at ultra-narrow furrows and high stocking densities.

INDEXES	DS (cm)	Plants (m^{-2})	SAMPLING 1° 0-74	2° DDS 74-94	3° 94-136
RAF (cm^2g^{-1})	75	8	51.3a	36.7a	16.0a
	50	10	53.4a	38.6a	14.2a
	35	12	51.8a	36.9a	13.8a
AFE (cm^2g^{-1})	75	8	117a	116.1a	116.1a
	50	10	117a	116.1a	116.1a
	35	12	118a	116.1a	116.1a
RPF (gg^{-1})	75	8	0.43a	0.31a	0.13a
	50	10	0.45a	0.33a	0.12b
	35	12	0.44a	0.31a	0.12b
Similar letters are statistically equal DMS (P≤ 0.05)					

Table 5 Growth rates of conventional cotton Fiber Max 989 at ultra-narrow furrow planting and high population densities

Conclusions

The highest yield was obtained in furrows at 35 cm and a stocking density of 120,000 plants ha-1. Fibre quality was not affected by row spacing or stocking density.

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Row spacing did not affect most of the growth rates determined. Except for the distance of 75 cm, which obtained better values in RPF than the other distances, thus a greater magnitude of the photosynthetic apparatus in these plants, and a greater efficiency in the distribution of photoassimilates.

The 35 cm furrow production system is an alternative to increase yield, reduce production costs, without reducing quality.

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Use of rapid test glycoproteins associated with pregnancy and ultrasound in early diagnosis of pregnancy in cow

Uso de la prueba de glucoproteínas asociadas a la gestación y la ecografía en el diagnóstico precoz de la gestación en bovinos productores de leche

GARCÍA-GOZÁLEZ, Daniela, ALARCÓN-ZAPATA, Marco Antonio, GARCEZ-MERCADO, Nora and TABAREZ-ROJAS, Abigail*

Facultad de Ciencias Biológicas y Agropecuarias, Universidad Veracruzana. Tuxpan, México. Facultad de Ciencias Biológicas y Agropecuarias de la Universidad Veracruzana. Carretera Tuxpan-Tampico Km. 7.5, Colonia Universitaria, Tuxpan, Veracruz, México, CP 92895. Tel. 017838344350 ext. 46109.

ID 1st Author: *Daniela, García-González* / **ORC ID** 0009-0009-6590-6587

ID 1st Co-author: *Nora, Garcez-Mercado* / **ORC ID** 0000-0002-4712-4663

ID 2nd Co-author: *Marco Antonio, Alarcón-Zapata* / **ORC ID** 0000-0002-4712-6327, **CVU CONAHCYT ID**: 176712

ID 3rd Co-author: *Abigail, Tabarez-Rojas* / **ORC ID** 0000-0002-8766-6993, **CVU CONAHCYT ID**: 176667

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Abstract

The study was carried out with the objective of determining the efficiency of pregnancy-associated glycoprotein test compared to ultrasound. 146 cows of the Holstein Friesian breed were used. The early diagnosis of pregnancy was made with the ELISA test at 28 days post-breeding and the first confirmation by ultrasound on day 35 and day 50 post-breeding a second confirmation. Statistical analysis was performed with chi-square test to determine the differences between the diagnostic test (rapid test and ultrasound). Differences were observed (P=0.009) between the two diagnostic tests, 68.5% of pregnant animals were detected with the blood test and 47.3% with ultrasound, with respect to ultrasound 1 and 2, no statistically significant differences were observed (P=0.1). The blood test allows the early diagnosis of cows that were not pregnant, and, in this way, they can be reincorporated into reproductive management practices, reducing the period of days open.

Glycoproteins associated with pregnancy, Ultrasound, Cow

Resumen

El estudio se realizó con el objetivo de determinar la eficiencia de la prueba de glucoproteínas asociadas a la gestación en comparación a la ecografía. Se utilizaron 146 vacas de la raza Holstein Friesian. Se realizó el diagnóstico precoz de gestación con la prueba ELISA a los 28 días post-servicio y la primera confirmación mediante la ecografía al día 35 y posteriormente al día 50 una segunda confirmación. El análisis estadístico se realizó con la prueba chi cuadrada para conocer las diferencias entre las pruebas diagnósticas (Prueba rápida y Ecografía). Se observaron diferencias (P=0.009) entre las dos pruebas diagnósticas, se detectó el 68.5% de animales gestantes con la prueba en sangre y 47.3% con la ecografía, respecto a la ecografía 1 y 2 no se observaron diferencias estadísticamente significativas (P=0.1). La prueba en sangre permite el diagnóstico temprano de vacas que no quedaron gestantes y de esta manera pueden ser reincorporadas a las prácticas de manejo reproductivo, reduciendo el periodo de días abiertos.

Glucoproteínas asociadas a la gestación, Ecografía, Vacas

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* Author Correspondence (Email: atabarez@uv.mx)
† Researcher contributing as first author.

Introduction

For years reproduction has been an important factor for large dairy producers, with new technologies in the dairy industry the greatest objective is to take advantage of all types of opportunities, animals producing high quantities of milk and producing offspring, as well as reducing the parameter open days and economic losses (Lee, 2007). Currently, the reduction of open days in a dairy herd constitutes one of the biggest challenges for veterinary technicians or doctors in order to obtain high reproduction parameters.

The duration of gestation is 283 days (243-316 days) and can be divided into an embryonic period, which runs from fertilization to 45 days, and a fetal period, from 46 days to delivery. The duration of gestation is influenced by maternal, fetal, genetic and environmental factors (Hernández, 2016).

One of the great obstacles for large production units are abortions, which is the expulsion of a fetus with some pathology, sometimes immature (premature). From 20 to 25 percent of cows diagnosed as pregnant lose pregnancies after day 45 of service. Like embryonic death, if it dies in the first 18 days of pregnancy it is considered an early embryonic death: when this happens, it can be observed that the cow presents estrus from days 21 to 24 after breeding (Wiltbank *et al.*, 2016).

If the embryo dies between days 24 to 42 (before organogenesis is completed), it is considered a late embryonic death; when this happens in cows, intrauterine resorption of the embryo occurs and a delay in the appearance of estrus is observed. But if the death of the embryo occurs after day 45, it is considered fetal death (Diskin and Morris, 2008; Hernández, 2016).

When the embryos or fetuses die in these cases, they are very small, and this goes unnoticed in the pens due to the size in which they are found and, in these situations, the cows only return to estrus. Producers limit themselves to days 28 and 30 of gestation as the closest point for accurate diagnosis of pregnancy due to the effectiveness of ultrasound and chemical methods using pregnancy-associated glycoproteins that are commercially available.

Interferon-tau (IFNτ) is taken as a sign of early pregnancy since it prevents luteolysis of the corpus luteum (CL), by directly or indirectly blocking the synthesis of oxytocin receptors in the endometrium and consequently the pulsatile production of PGF2α (Hernández, 2018).

The detection of pregnancy-associated proteins (PAG) using ELISA is considered a sensitive and accurate alternative for the diagnosis of early pregnancy in cattle, in addition to being non-invasive, therefore the objective of the study was to compare the efficiency of the test for proteins associated with pregnancy and ultrasound in the early diagnosis of pregnancy in dairy-producing cattle.

Material y methods

Location of the study area

This study was carried out on a farm in Actopan, Hidalgo, at an altitude of 2260 meters above sea level, with a semi-dry temperate climate, its minimum temperature ranges between 5.3°C reaching a maximum of 24.4°C (INEGI, 2017).

Experimental design

Multiparous Holstein Friesian breed cows (n=146) were used. The cows were stabled and milked twice a day. Pregnancy diagnosis was made in all cows by means of the rapid visual test for detection of glycoproteins associated with pregnancy in blood serum and by rectal ultrasound.

To perform the rapid visual test and detect pregnancy-associated glycoproteins (PAG), on day 28 post-breeding, a blood sample (approximately 8 ml) was taken from each cow by puncture of the coccygeal vein, using a caliber needle 21g and a vacutainer tube without anticoagulant. The blood samples were centrifuged at 3900 rpm for 5 minutes to obtain the blood serum and process it by enzyme immunoassay (ELISA) with the Bovine Pregnancy Test from the IDEXX laboratory, following the manufacturer's instructions. The presence of PAG was determined when the samples turned blue like the positive control.

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Pregnancy diagnosis by ultrasound was performed on all cows at 35±7 days post-breeding and was reconfirmed 15 days later. A Chison ECOVET-2 portable ultrasound machine with a 6 MHz real-time linear transducer was used. A female was considered pregnant when the amniotic vesicle was visualized, and the embryo's heartbeat was detected.

Statistical analysis

Data were analyzed with the Chi-square statistical test using the SPSS® 20 suite for Windows® (IBM SPSS, 2011).

Results

Of the 146 cows served, 100 pregnant cows were detected by the rapid test and 69 pregnant cows by the first ultrasound (figure 1), finding a significant difference for the diagnosis of pregnant cows (P=0.009).

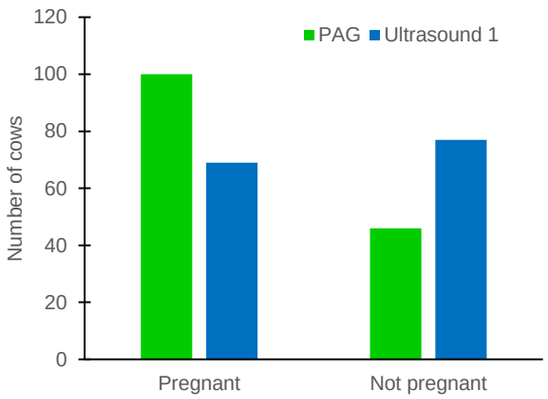

Figure 1 Diagnosis of pregnancy in cows using the rapid pregnancy-associated glycoprotein (PAG) test and first ultrasound.

The results obtained in the diagnosis of pregnancy through ultrasound in the second confirmation were maintained in 69 pregnant cows (Figure 2), which indicates that there were 31% embryonic losses in the period 35±7 days post-breeding according to the results obtained with the glycoprotein test associated with pregnancy in the period of 50±7 days post-breeding, without finding differences (P=0.1) in the diagnosis of pregnancy between ultrasound 1 and ultrasound 2, this means, that ultrasound is 21% more effective compared to the rapid test for glycoproteins associated with pregnancy, the latter being 69% effective.

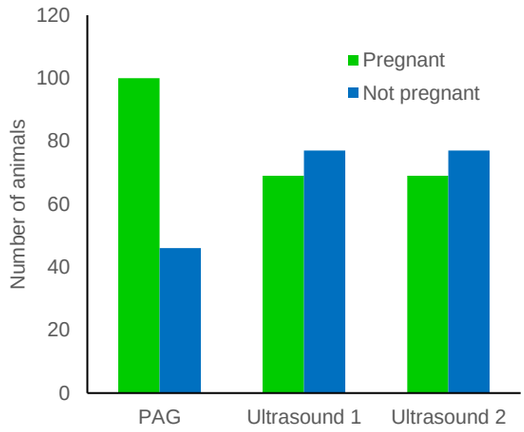

Figure 2 Number of pregnant cows according to the rapid pregnancy-associated glycoprotein (PAG) test, first ultrasound and confirmation ultrasound.

Table 1 shows the percentages obtained through the rapid pregnancy diagnosis test and ultrasound; for false positives, 21.24% and 0% were obtained respectively. The true positives for the pregnancy diagnosis were 69 animals, the ultrasound was 100% effective in determining pregnancy in the cows, however, with the rapid test there were 31 false positive animals that were considered embryonic losses because at the time of performing the test were pregnant, however, when the ultrasound was performed, they were no longer pregnant.

As for the true negatives for pregnancy, there were 46 animals for the rapid test and 77 animals for the ultrasound.

Diagnostic	Rapid test		Ultrasound	
	(n)	(%)	(n)	%
False positives	31	21.2	0	0
False negatives	0	0	0	0
True positives	69	47.3	69	47.3
True negatives	46	31.5	77	52.7
Total	146	100	146	100

Table 1 Percentage of false positives, false negatives, true positives and true negatives, through the rapid pregnancy-associated glycoprotein (PAG) test and ultrasound

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Discussion

The percentage of pregnancy with the rapid test for glycoproteins associated with gestation at 28 days post-breeding was 68.5%, while with ultrasound at 35±7 days and 50±7 days post-breeding, 47.3% were detected. of pregnant animals, there being a difference of 21.2% between both methods, which contrasts with what was observed by Hernández (2020), in cows of dairy breeds when carrying out the pregnancy diagnosis with both the ELISA IDEXX kit and with ultrasound, they determined that both methods are closely related because there is no variation between the results of the diagnostic tests. Similarly, Antelo and Ibáñez (2015), in beef breed cows when carrying out the diagnosis of pregnancy with the IDEXX rapid test and rectal palpation, did not find statistically significant differences in the percentage of pregnancy between both tests, on the contrary, Garate and Suárez (2015), when comparing the IDEXX rapid test at 28 days post-breeding and rectal palpation at 60 days post-breeding in milk-producing cows, determined a minimal difference between the diagnostic methods, determining 47.6% of gestation for the ELISA test and 40.7% for rectal palpation, pointing out that the ELISA method with the IDEXX kit is very precise and that the difference between diagnostic methods may be affected by embryonic death or skill of the personnel at the time of perform the diagnosis.

In this sense, in this study it was considered that the difference in the pregnancy results obtained between the methods for diagnosing pregnancy was due to embryonic losses (31%), since it is well known that during the first 60 days of pregnancy losses occur (Sice *et al.*, 2022) and in the event that there is an embryonic loss, the proteins associated with pregnancy remain circulating in the blood for 7 to 14 days after the loss (Humbolt, 2002), the half-life of trophoblastic proteins varies depending on the degree of glycosylation they present in their structure, although on average it is described as 7.2 days (Jerome, 2012). On the contrary, ultrasound is more accurate in detecting pregnancy by identifying the amniotic vesicle and heartbeat. However, as Garate and Suárez (2015) point out, the IDEXX kit helps identify empty cows early, allowing them to be reintegrated into new synchronization protocols, saving time and money.

Another aspect to consider in the results is the percentage of embryonic loss in the study (31%), this result is below what Diskin *et al*. (2012) reported for high-producing cows, the rate of embryonic and fetal loss ranges from 40 to 56% respectively. However, improving the embryonic survival rate remains the main challenge, given the antagonistic relationship between embryo production and survival.

Conclusion

The use of the protein test for early detection of pregnancy at 28 days post-breeding makes it possible to determine pregnant cows with 100% efficiency, helping to detect cows that are empty very early to reintegrate them into new protocols synchronization and/or artificial insemination or natural mating, reducing the interval of days open.

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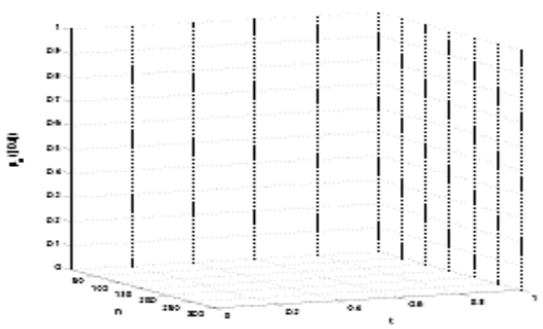

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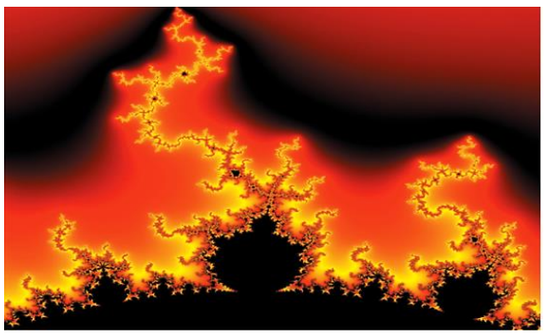

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The description of the peer review processes is made known by the Editorial Board in order that the Authors know what the evaluation criteria are and will always be willing to justify any controversy in the evaluation process. In case of Plagiarism Detection to the Article the Committee notifies the Authors for Violation to the Right of Scientific, Technological and Innovation Authorization.

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The Arbitrators undertake to notify about any unethical conduct by the Authors and to indicate all the information that may be reason to reject the publication of the Articles. In addition, they must undertake to keep confidential information related to the Articles they evaluate.

Any manuscript received for your arbitration must be treated as confidential, should not be displayed or discussed with other experts, except with the permission of the Editor.

The Arbitrators must be conducted objectively, any personal criticism of the Author is inappropriate.

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The authors have requested that the text in all its forms be an unethical editorial behavior and is unacceptable, consequently, any manuscript that incurs in plagiarism is eliminated and not considered for publication.

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Editorial Policy and Management

21 Santa Lucía, CP-5220. Libertadores -Sucre–Bolivia. Phones: +52 1 55 6159 2296, +52 1 55 1260 0355, +52 1 55 6034 9181; Email: contact@ecorfan.org www.ecorfan.org

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