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Knowledge Area

The works must be unpublished and refer to topics of Experimental design, commerce, options, rural-flora and fauna, agronomy, natural and other topics related to Biology and Chemistry.

Presentation of the Content

In the first article we present, *Green Synthesis of new heterocyclic hydrazones, spectroscopic characterization*, by CABRERA-VIVAS, Blanca Martha, PALILLERO-CISNEROS, Angel, MORALES-LARA, Laura, and MELÉNDEZ-BALBUENA, Lidia, with adscription in Benemérita Universidad Autónoma de Puebla, as following article we present, *Insulation of basiodiomicet fungies phosphorus solubilizers and nitrogen fixers for application as mycorrhizas in lechuga (Lactuca sativa)*, by GÓMEZ-VALADEZ, Alejandrina, JIMÉNEZ-GONZÁLEZ, Angélica, CONDE-BAEZ, Laura and GAYOSSO-MEXIA, Ana Rosa, with adscription in Universidad Politécnica de Pachuca, as following article we present, *Effect of the analogue of L-carnitine, β-hydroxyphosphocarnitine on the metabolism of Nocardia brasiliensis* by MARTÍNEZ-ROBLES, Sandra, GONZÁLEZ-BALLESTEROS, Erik, REYES-ESPARZA, Jorge and VARGAS-HERNÁNDEZ, Genaro, with adscription in Universidad Nacional Autónoma de Mexico, Universidad Autónoma del Estado de Morelos and Universidad Politécnica de Pachuca, as following article we present, *A review of the state of the art of feature extraction of electroencephalographic signals*, by ESQUEDA-ELIZONDO, José Jaime, JIMÉNEZ-BERISTÁIN, Laura, CHÁVEZ-GUZMÁN, Carlos Alberto and GUERRA-FRAUSTRO, Ricardo Jesús Renato, with adscription in Universidad Autónoma de Baja California, as following article we present, *Subprotestic stomatitis caused by incorrect choice of fixed protesis treatment*, by ROSADO-VILA, Graciella, SÁNCHEZ-ÁLVAREZ, Lucía, OROZCO-RODRIGUEZ, Angel Ruben and ORDOÑEZ-CHAVEZ, Guadalupe, with adscription in Universidad Autonoma de Campeche, as the last article we present, S*ynchronization of jealouses in simowal cows using Gnrh and PGF2α in the Conception, Michoacán*, by LUCIO, Rodolfo, SESENTO, Leticia, BEDOLLA, José Luis Carlos and CRUZ, Ángel Raul, with adscription in the Universidad Michoacana de San Nicolás de Hidalgo and Colegio Primitivo y Nacional de San Nicolás de Hidalgo.

Content

Article **ECORFAN Journal-Ecuador**
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Green Synthesis of new heterocyclic hydrazones, spectroscopic characterization

Síntesis vía Química Verde de nuevas hidrazonas heterocíclicas, caracterización espectroscópica

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Abstract

The present project analyses the synthesis of three hydrazones with new structures by Green Chemistry, for its later spectroscopic characterization. Hydrazones are organic compounds with interesting properties, such as being solids with specific melting points and colors depending on their structure. Hydrazones and substituted derivatives are a group of versatile compounds and leading molecules because they are potential bioactive agents with an ample spectrum of pharmacological activities. It is important to note that these properties are a consequence of the presence of the imine group that possess high electronic density. The objective is the synthesis of heterocyclic hydrazones derived from furan and thiophencarbaldehyde with novel structures that allow the synthesis of optimized compounds with biomedical applications. Synthesis, purification and characterization of organic compounds was designed to combat illnesses with maximum positive effects and minimum toxicity, in order to evaluate their activity in specific cell lines.

Green Chemistry, Hydrazones

Resumen

El presente trabajo es la síntesis de 3 hidrazonas de estructura nueva, con Química Verde, y posterior caracterización espectroscópica. Las hidrazonas son compuestos orgánicos, con singularidades, como de ser sólidos con puntos de fusión característicos, color determinado dependiendo de la estructura. Las hidrazonas y sus derivados sustituidos son un conjunto de compuestos versátiles considerados moléculas líderes, por ser agentes bioactivos potenciales y con amplitud de actividades farmacológicas. Cabe recalcar, que estas propiedades se deben principalmente a la presencia del grupo imino con alta densidad electrónica. El objetivo es la síntesis de hidrazonas heterocíclicas aromáticas derivadas de furano y tiofenocarbaldehído con nuevas estructuras que eficienten su síntesis, y contribuyan al desarrollo de estos compuestos con aplicaciones biomédicas. Se diseñó la síntesis, purificación y caracterización de compuestos orgánicos para combatir enfermedades con efectos máximos y toxicidad mínima, para evaluar su actividad en líneas celulares específicas.

Química Verde, Hidrazonas

Citation: CABRERA-VIVAS, Blanca Martha, PALILLERO-CISNEROS, Angel, MORALES-LARA, Laura, and MELÉNDEZ-BALBUENA, Lidia. Green Synthesis of new heterocyclic hydrazones, spectroscopic characterization. ECORFAN Journal-Ecuador. 2019. 6-11: 1-6

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Introduction

Hydrazones with an azomethine group –NHN=CH- represent an important class of compounds that possess a wide variety of biological activities. (Al-Hazmi, et al., 2019; Corey and Enders, 1976). It is believed that the azomethine group is essential for the bioactivity of hydrazones and derivatives. (Rollas and Küçükgüzel, 2007).

Specifically, hydrazones, class of organic compounds have attracted attention of medicinal chemists because of the azomethine group bonded to a carbonyl group, which is responsable for a variety of biological activities. Several papers around the world have reported hydrazone structures in search for better activity agents against different targets with lower toxicity (Rahmet, 2012). In recent years, it has been discovered the highest antibacterial activity of the structure hidrazide-hydrazone (El-Gammal, 2019; Popiolek, et al., 2017).

In the last decades the presence of infectous diseases is on the rise. The Center for Control and Prevention of diseases (CDC) has estimated more than 2 million infections and 23,000 deaths caused by bacteria resistant to antibiotics in the USA every year and 18 drug resistant pathogens, which are considered a threat (CDC, 2013). *Enterococcus faecium*, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species are considered ESKAPE pathogens (Rice, 2008), nothing that they escape from regular antibacterial drugs. Categories of this class of pathogens: urgent, serious and distressing (Chu, et al., 2019). This fact prioritizes the search for alternative strategies to overcome antibacterial resistance between bacterial pathogens (CDC, 2013).

0.7 billion deaths take place worldwide due to drug resistant pathogens. This number may increse to 10 million by 2050 if the actual tendencies continue (Xu, et al., 2019). In addition, the overuse of drugs against infectous and non-infectous diseases has caused resistance against pathogenic bacteria, weakening the success of medication (Chopra, et al., 2008; Gao, et al., 2018).

This is why, the search for antimicrobial agents is a neverendingly task, with a relevant importance to explore and develop new and different structures that allow enhanced biological activities (Rollas, 2007), to employ them in clinical applications, or as chelating agents (Wahben, 2019). Moreover, it is also important to explore environmentally friendly methologies for their synthesis.

Hydrazones are an important class of molecules used as drugs for their biological activities such as: analgesic, anthelmintics, anticonvulsant, antidepressive, antiinflammatory, antimalarial, anticancerous and antibacterial (Rahmat, 2012).

Some antibacterial drugs available in the market are furacilina, furazolidona, ftivazida, nifuroxazida, nirofurazona y nitrofurantoine, contain a hydrazone group (Popiolek 2017).

Hydrazone derivatives have been well studied because they have been used as important precursors in the construction of several heterocyclic ring reactions (Rollas y Küçükgüzel 2007).

With this evidence it is crucial to develop new antibacterial agents with an excellent activity against pathogens, which are resistant to existing drugs.

The high spectrum of biological activities combined with structural modifications and successful applications in clinic practice have inspired many researchers to the study and creation of a great deal of hydrazone derivatives.

Popiołek *et al*., obtained fifteen new hydrazide-hydrazones by condensation of hidrazide of isonicotinic acid with different aromatic aldehydes, confirming their structure by espectroscopic methods. All sinthetized compouds were subjected to *in vitro* antimicrobial essays against "Gram-negative" and "Gram- positive" bacteria and the corresponding fungus, *Candida spp*. Some sinthetized hydrazide-hydrazone compounds were discovered to be compounds with significant antibacterial activity and more powerful than chimiotherapeutic agentes commonly used.

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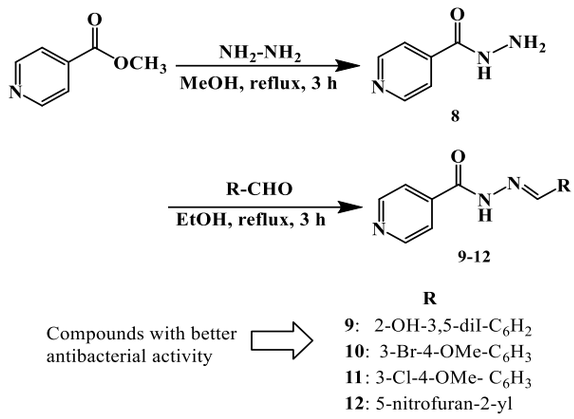


Scheme 1 Steps of the Popiołek synthesis of new acid isonicotinic hydrazide-hydrazones. Popiołek 2018

Data obtained by Popiołek *et al*. show that the new synthesized compounds 9-12, exhibit some sort of antimicrobial activity against reference bacteria and yeasts. Compounds 9 and 12 showed the highest antibacterial effect. Compound 9 exhibited a strong activity against all Gram-positive bacteria y bactericide effect towards *Staphylococcus aureus ATCC 6538*, *Staphylococcus epidermidis ATCC 12228* and *Bacillus subtilis ATTC 6633* and bacteriostatic effect against other bacteria. It is worthwhile mentioning compound 9 activity against *S. aureus ATTC 25923* and *M. luteus ATCC 10240*, which was 8 times and 32 times better than nitrofurantoine activity respectively.

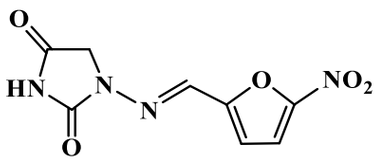


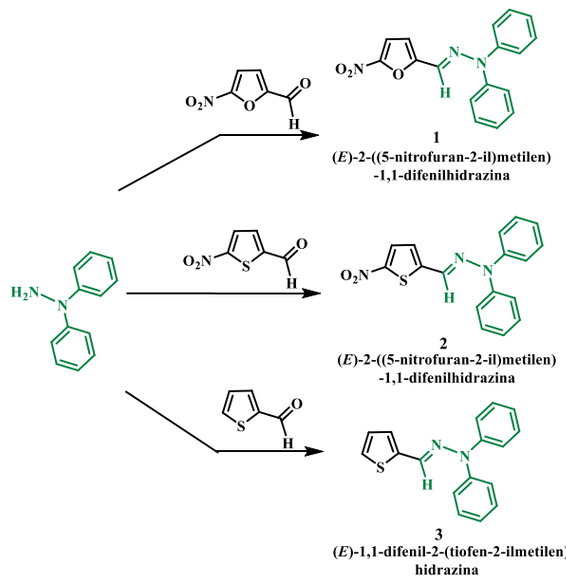
Figure 1 Structure of nitrofurantoin

The activity of this derivative was also significant against *B. subtilis* ATCC 6633, which was twice as high as cefuroxime activity and 8 times higher than ampiciline activity. This substance showed good bactericide activity against Gram- negative *Bordetella bronchiseptica* ATTC 4617. The activity against these bacteria was equal to the activity of nitrofurantoine with bactericide effect.

Compounds 10 y 11 showed moderate effect towards *M. luteus* ATTC 10240 and good bactericide activity against other Gram-positive bacteria. Moreover, compound 9 has a good antifungal effect towards *Candida spp*. (Popiołek, et al., 2018).

Objective

The main objective of this work is the synthesis, separation and purification of a hydrazone with new heterocyclic structure based on Green Chemistry, for its later characterization using spectroscopic technics such as ultraviolet–visible (UV–Vis), infrared (I. R.), hydrogen and carbon 13 magnetic resonance (^{1}H RMN, ^{13}C RMN), mass espectrometry (E. M.) and X-ray diffraction. Scheme 2 shows the synthesis under Green Chemistry of the aromatic hydrazones derived from the condensation reaction of 5-nitro-furanocarbaldehyde, 5-nitro-thiophenecarbaldehyde and thiophenecarbaldehyde each one with diphenylhydrazine.



Scheme 2 Synthesis under Green chemistry conditions of hydrazones 1, 2 and 3

Results

The hydrazone-type compounds shown in this work were synthesized from *N,N*-diphenylhydrazine with three different aromatic heterocyclic aldehydes derived from thiophenecarboxyaldehyde and furan-carboxydehyde. These reactions are carried out at room temperature and under constant stirring for several hours, depending on the reaction. The reactions were periodically monitored by thin layer chromatography. For the purification of these compounds they are allowed to crystallize and subsequently recrystallized by slow evaporation or diffusion.

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After having purified the products of the three reactions presented, their respective characterization was carried out by melting point determination by Tekno-Lab apparatus, UV-VIS spectra were recorded on on Varian Cary 50 Bio UV Visible, IR spectra were recorded on Nicolett FT-IR Magna 750, in KBr pellets, as the standard sample preparation technique, ^{1}H-NMR and ^{13}C spectra were obteined on a Varian VX-400 spectrometer, TMS was used as internal reference; mass spectrometry E.I. with elemental analysis were recorded on JEOL JEM JMS-SX 102 a 70 eV, and X-ray diffraction data was collected with an Oxford Diffraction Gemini "A" diffractometer equipped wih a CCD area detector (Agilent 2011 CrysAlis PRO). These studies were used to determine and corroborate the proposed structures of the three hydrazones 1, 2 and 3.

The results of the characterization of hydrazones are shown below.

E)-2-((5-nitrofuran-2-yl)methylene)-1,1-diphenylhydrazine (1). Reddish Orange scales; yield: 82% at 25 ºC, M. p. 136-138 ºC. UV λ_{max} = 440 nm. FT. IR (film): (cm^{-1}): 3136 ν(C_{sp2}-H) (furan), 3057 ν (C_{sp2}-H phenyl), 1558 ν(C=N), 1473 ν(C=C), 1342 ν(NO_2), 1215 ν(C-O furan), 754, 732 ν(C-H monosubstituted aromatic).^{1}H NMR (400 MHz, $(CD_3)_2CO$: (δ/ppm, *J*/Hz): 7.46 (dd, 4H, C3'), 7.39 (dd, 1H, C4), 7. 28 (dd, 2H, C4'), 7.19 (m, 4H, C2'), 7.00 (s, 1H, C=N), 6.82 (d, 1H, C3). ^{13}C NMR (400 MHz, $(CD_3)_2CO$): (δ/ ppm): 155.34 (C5), 151.12 (C1'), 142.13 (C2), 130.07 (C3'), 125.97 (C4'), 122.68 (C2'), 122.34 (C=N), 114.58 (C4), 108.53 (C3). MS-EI: 307.31 m/z = $C_{17}H_{13}N_3O_3$.

(*E*)-2-((5-nitrofuran-2-yl)methylene)-1,1-diphenylhidrazine (2). Bright red rhomboid scales; yield: 81% at 25 ºC, M. p. 128-130 ºC. UV λ_{max} = 465 nm. FT. IR (film): (cm^{-1}): 3024 ν(C_{sp2}-H thiophene), 2916 ν(C_{sp2}-H phenyl), 1587 ν (C=N), 1438 ν(C=C), 1483,1325 ν(NO_2), 700 ν(C-H (monosubstituted aromatic). ^{1}H NMR (400 MHz, $(CD_3)_2CO$: (δ/ ppm, *J*/Hz): 7. 78(d, 1H, C4), 7.46 (dd, 4H, C3'), 7. 28 (m, 2H, C4'), 7.19 (dd, 4H, C2'), 7.12 (s, 1H, C=N), 6.77(d, 1H, C3). ^{13}C NMR (400 MHz, $(CD_3)_2CO$): (δ/ ppm): 149.99 (C=N), 149.55 (C5), 142.28 (C1'), 130.04 (C3'), 129.38 (C4), 127.17 (C3), 125.76 (C2), 123.90 (C2'), 122.36 (C4'). MS-EI: 323.37 m/z = $C_{17}H_{13}N_3O_2S$.

(*E*)-1,1-diphenyl-2-(thiophene-2-methylene)hidrazine (3). Light yellow powder; yield: 70% at 25°C, M. p. 164-166 ºC. UV λ_{max} = 355 nm. FT. IR (film): (cm^{-1}): 3099 ν(C_{sp2}-H tiophene), 3061 ν(C_{sp2}-H phenyl), 1581 ν (C=N), 1494 ν(C=C), 756 ν(C-H (monosubstituted aromatic), 698 ν(C-S). ^{1}H NMR (400 MHz, $(CD_3)_2CO$: (δ/ ppm, *J*/Hz): 7.41 (m, 4H, C3'), 7.14(m, 7H, C=N, C2', C4'), 6.94 (dd, 1H, C4), 6.90 (m, 1H, C3). ^{13}C NMR (400 MHz, $(CD_3)_2CO$): (δ/ ppm): 143.28 (C2), 141.79 (C2'), 130.36 (C5), 129.80 (C3'), 127.13 (4), 126.29 (C3), 125.40 (C=N), 124.56 (C4'), 122.39 (C2'). MS-EI: 278.37m/z = $C_{17}H_{14}N_2S$.

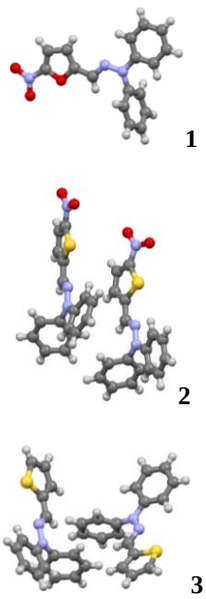

Figure 2 X-rays of the 3 synthetized hydrazones 1, 2 and 3

Discussion

Except for the synthesis of hydrazone **3**, the others are considered reactions with high yields. The melting point ranges obtained show that the hydrazones are pure. The observed colors of the hydrazones agree with the values obtained from the λ_{max} in the Ultraviolet spectrum. The values of the bands obtained in the Infrared spectrum show that the reaction was verified, when the carbonyl band (>C=O) was absent and that of the imine bond (>C=N), corresponding to the hydrazone. In NMR the imine hydrogen appears very constant, around 7.00 ppm for the 3 hydrazones, which means that it is not being affected by the presence or absence of the nitro group.

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Conclusions

The 3 hydrazones were obtained with good yields and under conditions of Green Chemistry, using the same chemical equivalents for reagents and product of each reaction, from this point of view, work was carried out on efficientizing and synthesizing hydrazones using an alternative and environmentally friendly method ambient. The structures of the hydrazones were characterized, coinciding in all cases with the expected structure (This copounds are stored keeping their high purity), which will promote their possible biomedical applications in subsequent studies as possible antiparasitic or antibacterial.

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Insulation of basiodiomicet fungies phosphorus solubilizers and nitrogen fixers for application as mycorrhizas in lechuga (*Lactuca sativa*)

Aislamiento de hongos basiodiomicetos solubilizadores de fósforo y fijadores de nitrógeno para su aplicación como micorrizas en lechuga (*Lactuca sativa*)

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Abstract

Poor agricultural practices have caused eroded soils, therefore alternatives are sought for the solubilization and fixation of nutrients by mycorrhizal fungi, that grow on the plants roots forming a mutualist symbiosis. Therefore, in this work, *Basidiomycetes* fungi were isolated and those that are able to solubilizing phosphorus and nitrogen were selected for their inoculation in lettuce (*Lactuca sativa*), this is high consumption and fast growth vegetable. Five substrates (fertile soil, eroded soil, humus and horse manure) were studied with a not inoculated control, where the response variable was hypocotyl growth and the *L. sativa* radicle, measured every 24 hours for 30 days. It was observed that the HM3 fungi stimulated the highest growth of the plant in eroded soil, generating an alternative to agriculture and contributing to bioremediation and exploitation of damaged soils.

Phosphorus, Nitrogen fixers, Mycorrhizae

Resumen

Las malas prácticas de agricultura han ocasionado suelos erosionados, por lo tanto se buscan alternativas para la solubilización y fijación de nutrientes mediante hongos mycorrhizal, los cuales crecen en las raíces de las plantas formando una simbiosis mutualista, por lo tanto en este trabajo se aislaron hongos basidiomicetos y se seleccionaron aquellos capaces de solubilizar fósforo y nitrógeno, para su inoculación en Lechuga (*Lactuca sativa*), este vegetal es de alto consumo y rápido crecimiento. Se estudiaron cinco sustratos (suelo fértil, suelo erosionado, humus y estiércol de caballo) con un testigo sin inocular, donde la variable de respuesta fue el crecimiento del hipocótilo y la radícula de *L. sativa*, medidos cada 24 h por 30 días. Se observó que el hongo que estimulo el mayor crecimiento de la planta fue el HM3 en suelo erosionado, generando una alternativa a la agricultura y contribuyendo a la biorremediación y aprovechamiento de suelos dañados.

Fosforo, Fijadores nitrógeno, Mycorrhizas

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Introduction

Mycorrhizas are non-pathogenic mutual symbiotic associations between fungi and plant roots (Turk et al., 2006). They are divided into endomycorrhizas (vesicle-arbuscular), these penetrate the root cells (Frey-Klett et al., 2007); and in ectomycorrhizas, which are characterized by forming outside the cortical cells of the root producing a structure called "Hartig's network" (Galindo-Flores et al., 2015). Among the most studied mycorrhizal fungi are arbuscular belonging to the genus Glomeromicota, which are characterized by participating in crops (Andrade and Silveira, 2008; Khaosaad et al., 2006).

On the other hand, ectomycorrhizas play a fundamental role in the development of woody trees such as pinus sp. (pines), Quercus sp. (oaks), etc. (Rúa et al., 2015). In this family are the fungi basidiomycetes and ascomycetes (Pierre-Emmanuel et al., 2010), on which few studies have been carried out to investigate the influence of these fungi on agricultural crops. In both cases (endo and ectomycorrhizas), the symbiosis is carried out because the fungi have a heterotrophic character and can obtain their carbon source from the metabolites provided by organisms such as plants, and in reciprocity they solubilize and fix the mineral nutrients and provide the soil water that their hosts need to grow (Honrubia, 2009).

Among the most important nutrients for plant development are nitrogen (N) and phosphorus (P), which limit growth. Different factors involved for nutrient availability are known, including pH, the amount of organic matter, humic and fulvic acids, citrates and oxalates (Rosatto-Moda, et al., 2014).

However, the assimilation of nutrients is carried out with the help of mycorrhizal fungi, since, by producing acidic substances such as lactic, citric, malic and gluconic acid, an ionization process of nutrients that are not directly available is initiated in the soil for vegetables (Katiyar et al., 2013). Due to these organic acids, NH4 is oxidized to assimilable nitrogen (Stavros et al., 2012), as is phosphorus, which is found as volcanic ashes or limestones and oxides of Fe and Al (Raij, 2011).

It has been determined that an appropriate interaction between soil-plant-fungus can provide farmers with a saving of 25 to 50% of fertilizers, since the nutrients existing in the soil are also used, in addition the crops have a greater development in terms of height, vigor and leaf area (Cruz-Hernández et al., 2014). Consequently, a 15 and 50% increase in fruit yields has been observed, leading to an alternative to sustainable agriculture (Noda, 2009).

Another benefit of this symbiosis is the protection against pathogens and a greater resistance to environmental stress (Nadeem et al., 2014), on the other hand, Vos et al., (2013) mention that mycorrhizas help fight some parasites such as nematodes, which cause rot of plant tissues.

The cultivation of vegetables for consumption is important, as they are a food that provide vitamins, minerals and fiber. Lettuce (Lactuca sativa) is one of the most consumed vegetables. In 2010, 340,383 tons were grown in Mexico, and its rapid growth facilitates laboratory study (SAGARPA, 2011).

However, due to poor agricultural practices and the abuse of chemical products such as fertilizers, herbicides and insecticides, a soil that is scarce in available nutrients has been generated, and their deficiency causes cultivated species to slow their growth or die. Therefore, in this work it is proposed to isolate phosphorus and nitrogen fixing basidiomycetes fungi, for mycorrhizal application in L. sativa using as substrate eroded and fertile soils.

Materials and methods

A random sampling of basidiomycete fungi was carried out in the town of Santa Monica, municipality of Epazoyucan in the state of Hidalgo, Mexico (19 ° -97 'North; 98 ° -61' West), during the rainy season in September. 2015. The fungi collected were subjected to an astringent treatment as indicated by Hine-Gómez and Abdelnour-Esquivel (2013). The stem was sectioned and 1 cm^3 segments were cut which were inoculated in papa dextrose agar (PDA) for seven days at 28°C.

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To select the fungi the samples were reseeded in culture medium yeast extract-mannitol-agar-blue bromothymol (ELMARC) for fungi with the ability to solubilize nitrogen (Angeles-Nuñez and Cruz-Acosta, 2015), and in Sundara medium and Sniha (SS) for phosphorus solubilizers. The selected fungi were reseeded in PDA medium and their spores were collected with 0.01% Tween 20.

To study the influence of fungi on the development of L. sativa, an experimental design of a factor with four levels and five replicates was used, using four substrates classified based on their type using pH and electrical conductivity (Salgado-Transit et al. al., 2011), which were designated as follows: fertile soil (SF), eroded soil (SE), humus (H) and horse manure (EC). To each substrate, five seeds of L. sativa were placed as indicated by Kim et al., (2010), 100 µL of a solution with 10^6 mL^{-1} spores of each of the selected fungi was inoculated (variable) Independent).

For each treatment a control was used without inoculation. As response variables, hypocotyl and radicle growth were measured every 24 hours for 30 days. To observe the effect of the microorganisms native to the soils used, the growth of L. sativa in Murashige and Skoog (MS) medium was also tested using the same response variables for 15 days. A statistical analysis of the results was carried out by means of an analysis of variance (ANOVA) and Tukey test, with the SPSS ™ 17.0 program.

At the end of this period, staining of the roots was performed on all treatments following the methodology described by Muñoz et al., (2009), in order to know if the isolated fungi generated a mycorrhizal symbiosis.

Results and discussion

25 different species of basidiomycete fungi were collected, of which three presented halos of solubility of both N and P, and were named as HM1, HM2 and HM3. The species with the highest solubility halo for nitrogen was HM3 and for phosphorus HM2.

This solubilization phenomenon occurs because the fungus secretes organic acids that modify the pH, for the ELMARC medium, the ammoniacal nitrogen is converted into assimilable nitrogen (NO_2, NO_3, and N_2), and in the SS medium the calcium phosphate present is ionized to PO_4^{3-}, which is a compound assimilable by the microorganism, depending on the amount of acid secreted is the size of the halo (Cordero et al., 2008; Cerón-Rincón and Aristízabal-Gutiérrez, 2012).

When performing the experimental design on the substrates selected with the fungi HM1, HM2 and HM3, it was observed that there was growth with the exception of horse manure, where both in the control group and in the experimental group there was no development of lettuce seeds during the 30 days of experimentation. This is due to the fact that there is a difference in pH and electrical conductivity (Table 1) far from the appropriate values for the growth of both the seeds and the microorganisms used (pH 6-7 CE 1.0-1.4 dS m^{-1}) (Zarazúa-Villaseñor et al., 2007, Carranza et al., 2009). In addition, horse manure was the substrate that had the highest salinity value, which indicates that water absorption is affected since an alteration in osmotic pressure is generated (Achilli and Childress, 2010).

Substratum	Initial pH	Initial CE (dS m^{-1})	Final pH	Final CE (dS m^{-1})
MS medium	6.8	1.16	5.6	0.54
Humus	8.36	0.78	7.09	0.34
Fertile soil	7.23	1.09	7.02	0.25
Eroded soil	10.19	2.21	8.43	0.79
Horse manure	10.55	3.36	8.65	0.86

Table 1 pH and EC of the substrates used in the inoculation of the selected fungi and L. sativa

As for the other substrates that were studied, Fig. 1 shows the hypocotyl measurements of the plants at 720 h. It was observed that in all cases (SE, SF and H) there is greater growth in the groups treated with the selected fungi compared to the control groups ($P < 0.05$). In addition, the greatest growth occurred in the soil eroded with the HM3 fungus, followed by fertile soil and humus. Checking that the characteristics of the substrates directly influence the promotion of the interaction between fungi and L. sativa in their growth and potentialization.

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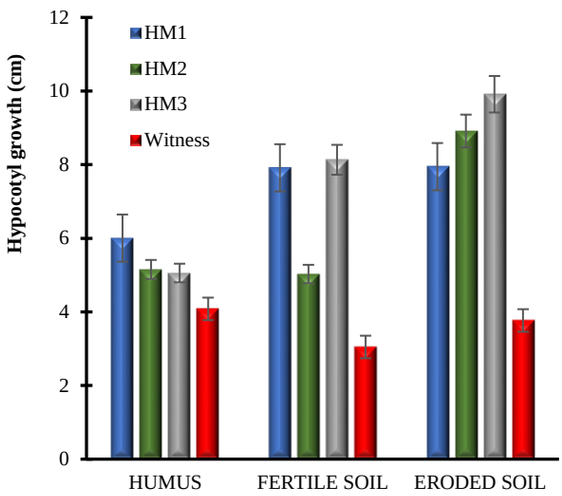

Figure 1 Growth of the hypocotyl of L. sativa at 720 h, with the different substrates and fungi selected

In addition to the above, in the group with the HM3 fungus, in addition to having the highest growth compared to the control group and the other experimental groups, it was observed that the appearance of the seedlings was of greater vigor (Fig. 2): the hypocotyl was thicker, larger leaves and the amount of seedlings obtained exceeded the number of inoculated seeds.

Therefore, the eroded soil is considered as the best substrate for the growth of L. sativa with isolated fungi, this symbiosis reflected an increase in the development of seedlings since it usually has a growth of 6.73 cm at 30 days (Terri-Alfonso et al., 2014), and with the HM3 fungus a length of 10.03 cm was reached in 30 days.

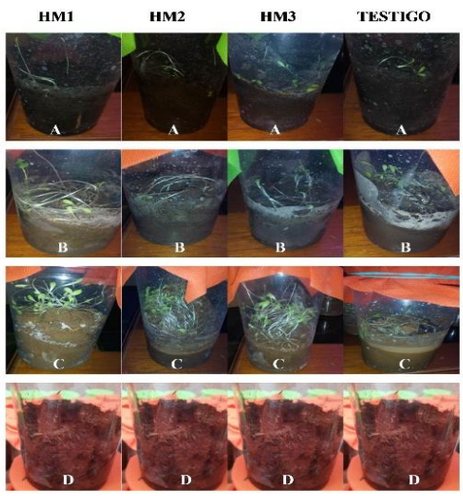

Figure 2 Sprouts of L. sativa at 720 h, on different substrates, with inoculated fungi.

Regarding the radicle, in Fig. 3 the development in the different substrates is shown at 720 h, in the analysis of variance a P <0.05 was obtained, and Tukey's analysis showed that the greatest growth in the investigated groups have HM2 in eroded Earth (3.0 cm). However, in general, there is no relationship between the addition of the isolated fungi and the length of the radicle, this with respect to the control groups.

However, it is recommended to make other types of measurements on the radicle, such as the root area and dry weight of this part of the plant since some differences were observed between the type of radicle developed in the control and experimental groups, suggesting that Fungi can help spread the root for better nutrient utilization (Noda, 2009).

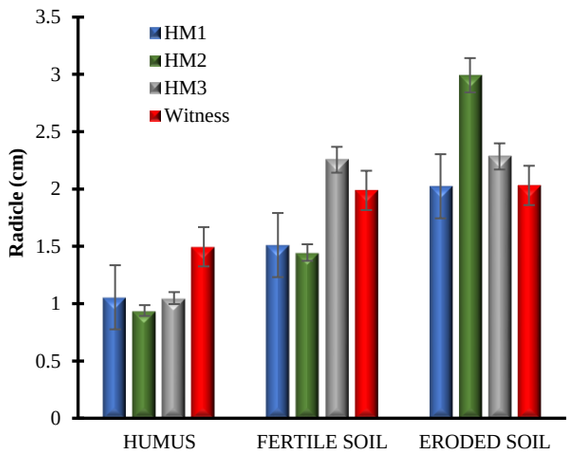

Figure 3 Growth of the L. sativa radicle at 720 h with the different substrates

In order to monitor the effect of the isolated fungi on L. sativa in a controlled environment and environments, the MS medium was used as a substrate and it was observed that the seeds with the fungi germinated at 48 hours and the control until the 120 hours.

This indicates that there is an effect when adding these microorganisms since they stimulate the growth of the embryo and this leads to the growth of the hypocotyl (Terry-Alfonso, 2014)

In addition the fungi adhered to the radicle of the plant observing the development of a symbiosis.

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During the treatment in the MS medium, which lasted 360 hours, a distribution and increase of the seedlings in the experimental groups was observed with the three fungi isolated as with the previous substrates, this phenomenon is known as potentialization because the When forming the association, they help plant species distribute the growth to obtain a greater amount of nutrients (Larkan and Smith, 2007), growing between 10 to 15 seedlings with the inoculated fungus and 5 seedlings in the control group (Fig. 4).

Figure 4 Test in MS medium with Lactuca sativa, at 360 hours of inoculation with the selected fungi (HM1, HM2 and HM3).

On the other hand, the three fungi studied generated a greater growth of the hypocotyl compared to the control at 360 hours (Fig. 5). ANOVA analysis showed that there is a significant difference in treatments compared to the control group ($P < 0.05$), however, there is no difference between the fungus MH1 and MH2 according to the Tukey test with a $P = 0.954$, with HM3 being the that stimulates greater growth.

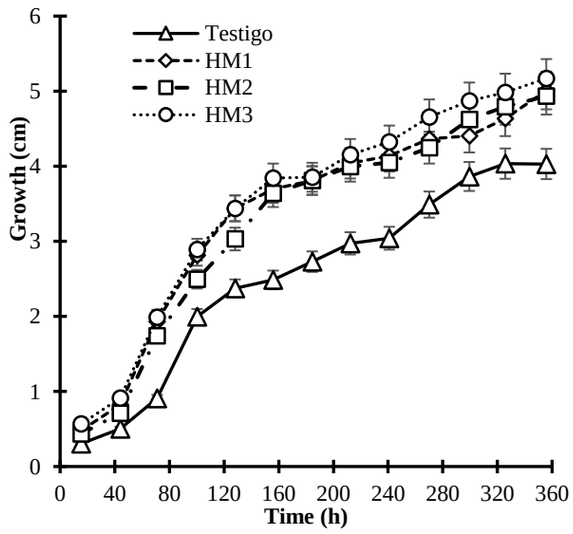

Figure 5 Growth of the hypocotyl of L. sativa inoculated with the selected fungi (HM1, HM2 and HM3)

Regarding the development of the radicle, it was determined that the control groups had a higher growth compared to the experimental groups (Fig. 6), this phenomenon of lower root development and higher hypocotyl growth with the fungus demonstrates that they produce substances bioactives capable of helping to increase nutrient fixation and thus stimulate the growth of L. sativa in the MS medium (González-Perigó et al., 2015), without having to develop a larger root surface.

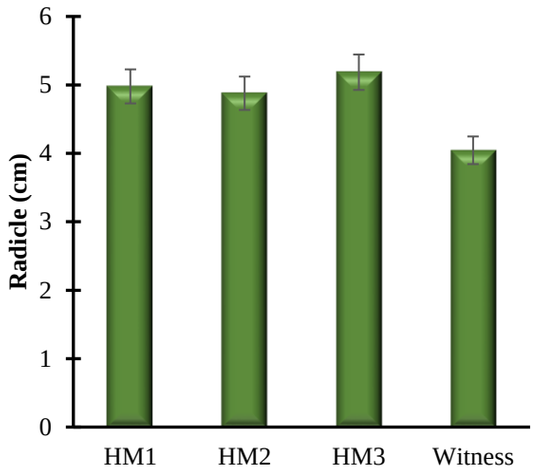

Figure 6 Growth of the L. sativa radicle, at 360 hours of inoculation with the selected fungi

Finally, the technique of clearing and staining on the roots was carried out (Muñoz et al., 2009) to check the presence of mycorrhizas, and it was observed that the isolated basidiomycete fungi formed a mycorrhizal symbiosis in the root cells of the plant unlike the control groups (Fig. 7).

This symbiotic behaviour has been observed in fungi of the Zygomycetes type such as Glomus sp., Which is one of the most studied fungi in this type of behaviour in order to increase the growth of some plants such as Zea mayz (maize) (Martín-Alonso et al., 2012), Solanum lycopersicum (tomato) (Mujica et al., 2014) Carica papaya (papaya) (Quiñonez-Aguilar et al., 2014), among others.

However, the promotion of a fungus of basidiomycete to mycorrhiza type in crops has so far been little studied.

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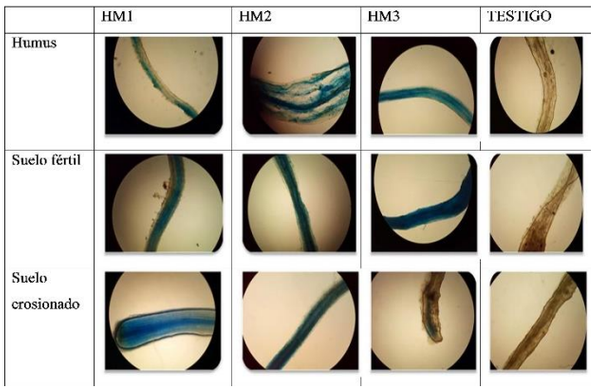

Figure 7 Root staining in L. sativa at 720 h growth with inoculated fungi, observed in the 400 X optical microscope

Finally, the positive effect of the promotion of mycorrhizal symbiosis of isolated fungi, especially of HM3 in L. sativa, suggests a potential use for its application in eroded soils, and with that in addition to increasing the production of this vegetable, it also I would be doing bioremediation indirectly.

This is possible because in this interrelation of species (fungus-plant), the plants select, attract and stimulate the microbial communities through exudates secreted by the roots (Cerón-Rincón and Aristízabal-Gutiérrez, 2012), and the Chemotaxis, in reciprocity, microorganisms stimulate growth through nutrient solubilization and production of bioactive substances (siderophores, antibiotics, hydrolytic enzymes and cyanidic acid) (Peña and Reyes, 2007).

Conclusions

Asylated basidiomycete fungi and selected phosphorus and nitrogen fixers are capable of forming mycorrhizal associations in L. sativa, they also provide a positive effect on the growth of said crop, observing that with the HM3 fungus the highest growth of hypocotyl was observed in soil eroded, this provides an alternative for sustainable agriculture and a contribution to the bioremediation of damaged soils.

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Effect of the analogue of L-carnitine, *β*-hydroxyphosphocarnitine on the metabolism of *Nocardia brasiliensis*

Efecto del análogo de L-carnitina, la *β*-hidroxifosfocarnitina sobre el metabolismo de *Nocardia brasiliensis*

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Abstract

Objectives: To determine whether β-HFC, in addition to its immunomodulatory effect as a therapeutic alternative in the combat of the mycetoma has an effect on the biochemical activity of *N. brasiliensis*. **Methodology:** determination of bacterial growth using N. brasilensis in the presence of β-HFC and biochemical analysis of its metabolism. **Contribution:** The immunomodulatory function of β-HFC in the treatment of chronic infections has recently been studied but it is not known whether it also has any antimicrobial effect, so in this paper the direct effect of β-HFC in *N. brasiliensis* will be elucidated in part.

Inmunomodulator, β-HFC, *Nocardia brasiliensis*

Resumen

Objetivos: Determinar si la β-HFC, además de su efecto inmunomodulador como alternativa terapéutica en el combate del micetoma tiene efecto en la actividad bioqímica de *N. brasiliensis*. **Metodología:** Se determinó el crecimiento bacteriano utilizando de *N. brasilensis* en presencia de β-HFC y análisis bioquímico de su metabolismo. **Contribution:** Recientemente se estudia la función inmunomoduladora de la β-HFC en el tratamiento de infecciones crónicas pero no se sabe si también tiene algun efecto antimicrobiano, por lo que en este trabajo se dilucidará en parte el efecto directo de β-HFC en *N. brasiliensis*.

Inmunomodulador, β-HFC, *Nocardia brasiliensis*

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Introduction

L-carnitine is a quaternary amine that in eukaryotes acts as a shuttle of long-chain fatty acids through the inner membrane of the mitochondria so that beta oxidation is carried out in the mitochondrial matrix (1), in bacteria, Carnitine is transported to the cytosol by the ABC system, or by the biotin / choline / carnitine transporter, in Gram positive and negative it is used by aerobic and anaerobic routes, as the final electron acceptor or as the sole source of carbon and nitrogen, to be catabolized to trimethylamine and malic semialdehyde, the latter will enter the cycle of tricarboxylic acids (2).

Beta-hydroxyphysphocarnitine (β-HFC), is an analogue of L-carnitine, which in addition to participating in lipid metabolism, lowers blood levels of glucose, cholesterol and increases circulating T lymphocytes (3), therefore β-HFC It is a good candidate to be an immunomodulator in infectious processes. Such as the mycetoma, chronic infection of the skin and underlying tissues with a tendency to affect bone, is characterized by a relatively painless increase in volume and fistulas through which pus and grains constituted by filaments are eliminated. In Mexico, the mycetoma is caused in 65% of cases by *Nocardia brasiliensis* (4), a Gram positive bacterium belonging to the phylum Actinobacteria, Cummins (5), Lechevalier (6) and Goodfellow (7), they place it Mycobacterium and Corynebacterium taxonomic group: all of them are distinguished by being aerobic, alcohol-acid variables, have branched mycelium or an aerial mycelium that tends to fragment, classification IV based on the chemotype of the wall and serology the walls of *N. brasiliensis*, *Mycobacterium tuberculosis and Corynebacterium diphtheriae types gravis and mitis* are closely related, since these genera cause diseases in humans and are rarely isolated from soils rich in decaying matter even when it is the natural habitat of actinomycetes (7).

The microbiological diagnosis of *N. brasiliensis*, long and thin filaments are observed in Gram staining, or forming clusters or tangles, are positive catalase, negative oxidase, use glucose, inositol and mannitol by oxidative route, hydrolyze casein, hypoxanthine, tyrosine and urea.

In addition they have in their genome other 32 proteases (8) Nocardia as a genus is biologically active, produces biomolecules by the route of synthesis of non-ribosomal peptides (NRPS) with three domains: A of adenylation, T of thiolation and C of condensation, domain A acts on an L-α-amino acid catalyzing the adenylation of the carboxyl end, in an ATP-dependent manner in a reversible classic ATP / Ppi reaction, (9). On the other hand, *in silico* studies of the lipid metabolism of said bacterium, it includes 15 acyl-CoA synthetases, 6 long-chain acyl CoA synthetases, 12 enoyl-CoA hydrostases / isomerases, 12 acetyl CoA acetyltransferases and a beta oxidation complex FadA / FadB (8) *N. cyriacigeorgica* for example, hydrolyses to petroleum-derived n-alkanes using an oxidative route from which alcohols, acid aldehydes and CoA esters are obtained with the subsequent beta oxidation (10), which demonstrates that these bacteria have developed strategies to produce surfactants or to join oils by hydrophobic bonds (11). Because the physiopathogenesis of *N. brasiliensis* depends on hydrolytic reactions that provide space and substrate in the host, it is necessary to know if the proteolysis and hydrolysis of fatty acids is susceptible to modulation by molecules that are used in immunomodulatory therapies so that The objective of this work is to determine whether, in addition to its immunomodulatory effect as a therapeutic alternative in the fight against mycetoma, β-HFC has an effect on the biochemical activity of *N. brasiliensis*, on the synthesis of biotechnological contribution molecules.

Methodology

Determination of antimicrobial activity by minimum inhibitory concentration (MIC)

To determine if β-HFC has an antibiotic effect, the minimum inhibitory concentration (MIC) and agar diffusion were evaluated, sulfamethoxazol-trimethoprim was used as a control antibiotic against *N. brasiliensis*, in both methods the concentration 62-64 µg / mL. Gram positive bacteria strains Staphylococcus aureus, Rhodococcus equi, *N. brasiliensis* FM-825, Nocardia brasiliensis HUJEG-1 ATC700358 and Escherichia coli were used as Gram negative, purity was verified according to Cowan and Steele's methodology (14), Conventional microbiology material was used, at a temperature of 25° C.

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For tests with *N. brasiliensis* and Rhodococcus equi, brain and heart infusion broth (BHI) agar, sheep blood agar, Dibico ©, for the other bacteria were used Müeller-Hinton Dibico © (MH) broth and agar was used, the Mc Farland turbidity standard 1.5x10^8 bacteria / ml (tube 0.5) was used and for the challenges the pure sulfamethoxazole salt (Sigma®) was used and the formula Sulfamethoxazole-Trimethoprim injection (Agro-Vet® Laboratories), β-HFC was provided by Nucitec © laboratories

Preparation of work solutions

320µg of sulfamethoxazole, solubilized in 2 ml of dimethylsulfoxide (DMSO) and graduated to 5ml, the working concentration was 64 µg / ml; 320µg of β-HFC were solubilized in 5 ml of PBS with the same working concentration, both solutions were sterilized by filtration with low bonded membrane and cooled until use.

In a 24-well plate, 1000 µL were dispensed in each of them, in well A1, 1000 microliters of the antimicrobial solution was served at the concentration indicated in document M-24-A, starting from this well obtained a 1: 2 concentration, 10 double dilutions were made, well B-5 was growth control (+) and well B-6 as sterility control (-) as shown in figure 1.

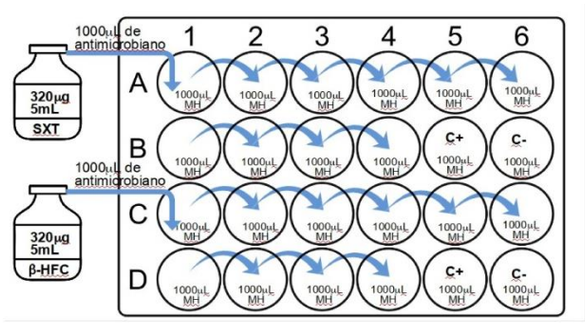

Figura 1 Preparation of strains and bacterial suspension for the MIC

In 50 mL of BHI broth, an inoculum of *N. brasiliensis* was seeded, after 7 days from the surface of the broth the growth film was taken, the bacterium was washed with PBS and disintegrated in Ten Broeck macerator until an orange suspension was obtained sui generis smell. The other bacterial strains were seeded in broth and M-H agar and manipulated according to microbiological techniques of antimicrobial susceptibility testing.

A suspension was adjusted to the turbidity standard 0.5 of the Mc Farland 1.5X10^8 nephelometer (150,000,000 CFU / mL), hence 0.5 mL was taken and 4.5 mL was added to a PBS tube (1:10 dilution), 1.5X107 (15,000,000 CFU / ml) 4 mL of the previous suspension was added to 36 mL of MH broth (1:10 dilution) 1.5X10^6 (1,500,000 CFU / mL)

10 microliters were served in 100 microliters of the bacterial suspension (1:10 dilution) 1.5X 10^5 (150,000 CFU / mL) per well of a 96-well plate (12 wells for this work modifying the quantities but retaining the concentrations) was dispensed in each of the wells of the plate as shown in Figure 2.

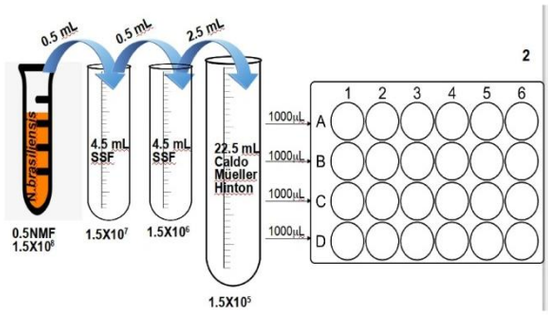

Figure 2 Microbial susceptibility testing protocol

From the suspension adjusted to 0.5 of the Mac Farland standard, a swab was taken and massively seeded in blood agar plate, commercial unidisks (Biorad) were impregnated with sulfamethoxazol-trimethoprim (STX), erythromycin 15 µg (E 15)) and dicloxacillin 1µg (DC), for the β-HFC were made well os with punch in agar and / or filter paper discs (64 µg / mL).

Determination of plate hydrolytic activity

From a solution of *N. brasiliensis* adjusted to 0.5 of the Mac Faland nephelometer in PBS plus β-HFC with a concentration of 64 mg / mL, in tween 80 agar plates, egg yolk, casein and tyrosine, prepared according At the indications of Cowan & Steele's (12) and López Martínez (13), 10 µL of the bacteria were sown in the media, in addition a batch of control group was prepared where *N. brasiliensis* was not in contact with the β-HFC. media were seeded the same day and allowed to grow at 25 ° C for 7 days.

The hydrolysis was developed with congo red or with lugol, the indicator was poured on the surface completely covering the agar, it was allowed to interact with the medium for 15 minutes, after time it was removed, the excess was rinsed with sterile SSF and dried to room temperature.

To obtain the power index, the diameter of the growth (A) and the diameter of the hydrolysis (B) were measured and B / A divided, the same experiment was performed but in broths with the substrates, plus β-HFC at a concentration of 64 μg / mL, the total volume of the bacteria plus the substrate was 2 mL, the supernatants were recovered and centrifuged to release them from the bacterium. The absorbance was read on Tecan GeniOS®, DO plate reader. 405, 540, 595 and 620.

Results

β-HFC has no bacteriostatic or bactericidal effect

The bacteria used in the test both Gram positive and negative showed no problems in their growth when incubated in the presence of β-HFC, in fact in the first well where it is more concentrated, there was greater growth (Figure 3), same as was corroborated by the technique of Miles & Misra (14), where the standard 0.5 of Mac Farland gave 240,000 CFU against 720,000 CFU of a solution of *N. brasiliensis* plus 64 mg / mL of β-HFC, in addition the STX was chosen as Control antibiotic since it is the one indicated to treat people suffering from mycetoma against *N. brasiliensis*, surprisingly, the MIC and agar diffusion tests showed that the bacteria are resistant (Figures 4 and 5a) to this antibiotic with the exception of S aureus, which was sensitive to a concentration of 0.25 mg / mL, *Rhodhococcus* equi had intermediate sensitivity (Figure 5b).

Figure 3 Bacterial growth in the presence of increasing concentrations of B-HFC

Figure 4 Bacterial growth in the presence of increasing concentrations of B-HFC

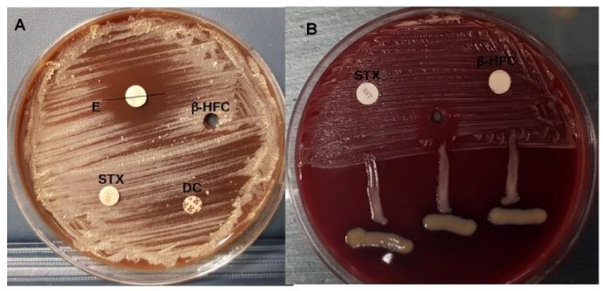

Figure 5 Babcterial growth by diffusion in agar. In the presence of B-HFC and STX. A) Nocardia brasiliensis, partial sensitivity (10 mm) to erythromycin, resistance to other antibiotics, B) Rhodococcus equi, partial sensitivity (10mm)

B-HFC increases the metabolic activity of *N. brasiliensis*

In the solid media with β-HFC the bacteria grew abundantly, and a difference was observed in the hydrolysis of the media with respect to the control, as shown in Figure 6.

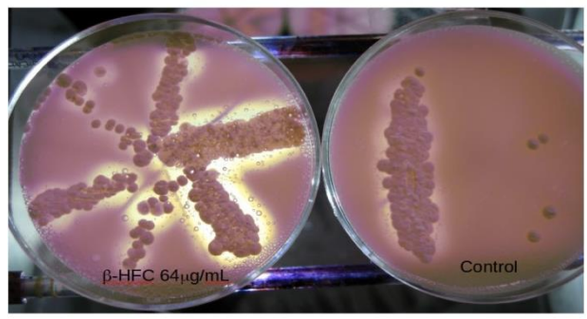

Figure 6 *N. Brasiliensis* hydrolytic activity in the presence of B-HFC (left) and control (right)

The power index was obtained, the results are shown in table 1, with all the substrates used for the growth of N. brasilensis the index was greater than 1 indicating that β-HFC stimulated the metabolism of the bacteria.

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Sustrato	Tratamiento	Crecimiento *(cm) (A)*	Halo de hidrólisis *(cm) (B)*	Índice B/A
Tirosina	*N. brasiliensis*	2	2.57	1.28
	N. brasiliensis + β-HFC	2	2.7	1.35
Caseína	*N. brasiliensis*	2	2.37	1.18
	N. brasiliensis + β-HFC	2	2.47	1.23
Yema de huevo	*N. brasiliensis*	2	2.23	1.11
	N. brasiliensis + β-HFC	2	2.3	1.15
Tween 80	*N. brasiliensis*	2	5.3	1.06
	N. brasiliensis + β-HFC	2	5.37	1.1

Table 1 Power index of substrate hydrolysis by *N. Brasiliensis*

The supernatants recovered from the broth hydrolysis also showed some differences, Figure 7 shows the graphs of the quantified absorbances analyzed by an ANOVA, a significant difference was observed, which is checked in the graph of pairs where tween 80, yolk of Egg and tyrosine show significant differences between them. As shown in the graph boxes and mustaches (figure 7A) and pairs (figure 7B). Additionally, a viable account was made, and it was confirmed that β-HFC has a growth activity of the bacterium by the amount of CFU counted (Table 2)

Sustrato	Estándar UFC/ml	Control UFC/ml	β-HFC UFC/ml
Tween 80	$2.4X10^5$	$4.58X10^6$ ± (1.2)	$1.1X10^7$ ±(1.2)
Caseína	$2.4X10^5$	$9X10^4$ ± (8.4)	$9X10^5$ ± (6.7)
Tirosina	$2.4X10^5$	$5.88X10^6$ ± (2.3)	$7.46X10^6$ ± (9.0)
Yema de huevo	$2.4X10^5$	$7.8X10^5$ ± (1.2)	$1.73X10^5$ ± (4.5)

Table 2 Colony forming units (CFU) of N. *brasiliensis* + β-HFC

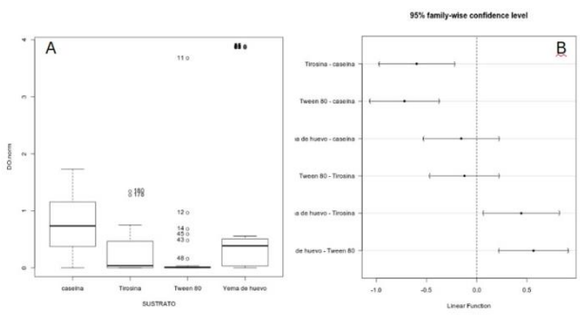

Figure 7 Quantification of the hydrolysis of N. Brasiliensis on different substrates a. Measurement of optical density. B. Peer quantification

Discussion

A bactericidal or bacteriostatic activity for L-carnitine has not been reported, it is used in weight and height control in obese people (15), and lately the immune response (16) has taken an important role in modulation, has studied its activity in tuberculosis, a chronic infectious disease of great importance worldwide, a report mentions an "antibacterial" effect of carnitine when administered orally, which resulted in an increase in the activity of CD4 + and CD8 + T lymphocytes possibly due to the increase in ATP that carnitine contributes to metabolism (17). In this work a carnitine analogue was used, and it was found that not only does it not have any antimicrobial activity, but it can also be used as a source of carbon and nitrogen (2). Because *N. brasiliensis* has a hydrolytic metabolism which causes chronic infections and remains viable for a long time, it is possible to think that it may not be appropriate to use carnitine as a therapy against it, perhaps the bacteria is able to carry Just β oxidation, according to the results obtained, substrates rich in fatty acids (tween 80 and egg yolk) and with amino acids (casein and tyrosine) were used for the proteolysis analysis of *N. brasiliensis* on the same over said compounds, Results obtained qualitatively speaking with the β-HFC analogue indicate that if the hydrolysis of the substrates increases (table 1), for the verification of significance in the difference, the analyzes were performed in visible spectrophotometry, the results show significant difference in the substrates rich in fatty acids (figure 7B), which suggests that the bacterium performs β oxidation and that it also allows mul type your number. The results obtained show that β-HFC does have an effect on the metabolism of *N. brasiliensis*, the main effect is on the increase of CFU, so carnitine favors both bacteria and T lymphocytes (2,17- 19).

Conclusions

Many questions arise with the findings, if carnitine favors both bacteria and T lymphocytes, will the bacteria and the immune system have to compete in a mycetoma infection? If the bacterium is stopped being treated as a pathogen and included as a biotechnological entity, if the administration of β-HFC favors the synthesis of biomolecules, can they be used against it to eliminate the microtome in patients suffering from it?

Elucidate the metabolic pathway that is induced to confirm that the β-HFC effectively activates the beta oxidation of *N. brasiliensis*.

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A review of the state of the art of feature extraction of electroencephalographic signals

Revisión del estado del arte de la extracción de características de señales electroencefalográficas

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Abstract

We present a review of the state of the art of the techniques and algorithms most used in the selection and detection of characteristics of electroencephalographic signals of people when consciously performing activities. These features are numeric parameters that describe the behavior of the signal and are the basis of patterns. In addition, previous experiences in the acquisition of electroencephalographic signals using the Epoc brain-computer interface manufactured by Emotiv are presented. First, some techniques used to eliminate artifacts (disturbances) present in the signal generated by blinking, strong breathing or other movements that contaminate the signal are presented. Later, the algorithms most frequently used in the processing of electroencephalographic signals are shown for the extraction of characteristics that describe the behavior of these patterns and that can be used to detect and recognize patterns in other signals. Finally, we present the lessons that we have acquired as a work team in the recording of electroencephalographic signals in order to be helpful for beginners.

Feature extraction, Electroencephalographic signals, BCI

Resumen

Se presenta una revisión del estado del arte de las técnicas y los algoritmos más empleados en la selección y detección de características de señales electroencefalográficas de personas al desarrollar actividades de forma consciente. Estas características son parámetros numéricos que describen el comportamiento de la señal y son la base de los patrones. Asimismo, se presentan experiencias adquiridas en la adquisición de señales electroencefalográficas con la interfaz cerebro computadora Epoc del fabricante Emotiv. Se presentan primero técnicas empleadas para eliminar artefactos (peturbaciones) presentes en las señales generadas por parpadeos u otros movimientos que contaminan a la señal. Posteriormente se muestran los algoritmos más usados en el procesamiento de señales electroencefalográficas para la extracción de características que describan el comportamiento de esos patrones y que puedan ser utilizados para detectar y reconocer patrones en otras señales. Finalmente, se presentan las lecciones que como equipo de trabajo hemos adquirido en la toma de registros de señales electroencefalográficos, esperando que esto sea útil a los que inician en el campo.

Extracción de características, Señales electroencefalográficas, BCI

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Introduction

With the appearance of a variety of commercial Brain Computer Interfaces (BCI) systems, there has been a need to carry out research in the EEG pattern detection. These BCI systems can be used in applications for medical, smart environments, detection, rehabilitation, gaming, education, authentication and other areas (Abdulkader, Atia, & Mostafa, 2015). This paper has the intention to help other people that are beginning in EEG signal processing. BCI systems use electroencephalographic (EEG) sensors (electrodes placed on the scalp) to acquire these signals and send them to an external device where it can be transformed into instructions or commands. Generally, a BCI system is divided in the following processes: signal acquisition, pre-processing, feature extraction and classification (Miao, Wang, Zhao, & Liu, 2016). The key factors that define the effectiveness of a BCI system are the feature extraction and the classification stages.

Signal acquisition

Many BCI systems are commercially available, like those presented in (Ramadan & Vasilakos, 2017). The BCIS are made with one to 256 electrodes and there are wired or wireless. There are open source BCIs like OpenBci or others like Epoc Emotiv, that require a license. Also, in (Ramadan & Vasilakos, 2017) some software used with BCIs or for signal analysis are presented. With this software some preliminary signal analysis can be done.

Pre-processing

The preprocessing stage generally includes eliminating the isoelectric lines of each channel, filtering and converting data from integer to a floating point that represents the real voltage level measured. Using digital signal processing techniques, it is possible to reduce noise, interference and the artifacts present in the signal. This must be done before the feature extraction stage. This procedure is shown in figure 1.

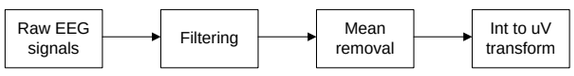

Figure 1 Signal preprocessing
Source: Self-Made

Features

The features are numeric parameters used to describe signals, in this case, the EEG signals. Some common features are: amplitude of the EEG signals, band power, power spectrum density, autoregressive parameters, time-frequency features and features based in inverse models.

Feature considerations

When a BCI system is designed, there are some points that need to be considered:

– Noise and atypical values: EEG signals have noise and they have atypical values because of the low signal to noise ratio.
– High dimensionality: feature vectors normally have high dimensionality because the come from different channels and from several time segments before they can be concatenated in only one feature vector.
– Time information: the extracted features have time information like the patterns related to some variations in a specific time window of the EEG.
– Non stationarity: EEG signals are non-stationary because they change rapidly with respect to time.
– Small training sets: datasets are relatively small because the training process requires time and data.

EEG time variations considerations

Most of the brain activity patterns used in a BCI system are related to particular time variations of the signal, possibly in specific frequency bands. For this reason, it is necessary to consider the timeline during the feature extraction stage, (Lotte, Congedo, Lécuyer, Lamarche, & Arnaldi, 2007) propose:

– Concatenation of different time segments features: this consists of feature extractions from different time segments and concatenating them into one single features vector.

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– Combination of classificatory algorithms in different time segments: this consists of making the feature extraction and the classification in different time segments and then combining the results of the different classifiers
– Dynamic classification: this consists of extracting features from different time segments in order to construct a temporal sequence of feature vectors.

Feature extraction

Feature extraction allows us to obtain useful or descriptive information hidden in a signal by decreasing the unnecessary or redundant information. In the feature extraction stage, some algorithms are used in order to obtain the useful information hidden in the signal that describe its behavior. It usually involves a dimensionality reduction or a data compression process that also reduces the amount of data needed to process. The feature extraction evolved from time processing (1960s), frequency processing (1960s-1980s), time-frequency (1980s-2000+) and sparse (2000+-today) (Krishnan & Athavale, 2018). With this classification, the feature extracting techniques can be grouped in:

– Time domain
– Frequency domain
– Joint time – frequency domain
– Signal decomposition and sparse domains

Time domain feature extraction techniques

This kind of techniques extract characteristic properties or features for a specific time window containing N samples. As we know, biomedical signals are non-linear and non-stationary, so the time window can be placed anywhere. The underlying patterns or descriptive information could remain the same for specific phenomenon presented in the EEG signals. The basic features that can be extracted are their statistical properties, such as mean, variance, standard deviation, quadratic mean value, etc. There are also some other feature extraction time domain techniques, such as cross correlation, Autoregressive modelling (AR), Linear Predictive Coding (LPC), Cepstrum analysis and Kernel based modelling.

AR modeling and LPC calculate the future values as a function of the current and past values. The Cepstrum analysis is based on the rate of change of different frequency spectrum bands of the analyzed signal. It is similar to the homomorphic filtering, where the signals are transformed by joint additions and multiplication operations. One of the advantages of the AR modelling is that it makes an enhanced data compaction and for spectral peaks detection, and also reduces signal noise and it provides a very good resolution. The order of the AR model cannot be determined a priori.

Cepstrum does not need to calculate the Fourier Transform of the signal, so it is considered a time domain processing. Cepstrum requires that the analyzed signal must be stationary over the time interval. This technique has been used in seismic, voice and geo-physical signals.

Frequency domain feature extracting techniques

These techniques transform the signals from time domain to frequency domain in order to extract low level features of a windowed signal. But sometimes these characteristics may not represent the real stationarity of the signal and only obtain global information that approximately classifies the signals.

Power Spectrum Density

The power spectrum density (PSD) function shows how the power of a signal is distributed in the frequency. This can be obtained with the Wiener-Khinchine Theorem or with the Short Time Fourier Transform. The Wiener-Khinchine Theorem express that the PSD and the Correlation are Fourier Transform pairs, as it is shown in

$$S_{xx}(f) = \Im\left[R_{xx}(\tau)\right] \quad (1)$$

$$R_{xx}(\tau) = \Im^{-1}\left[S_{xx}(f)\right] \quad (2)$$

Where *Sxx(f)* is the self-spectrum or PSD of the signal *x(t)* and *Rxx(τ)* is the autocorrelation of the signal *x(t)*. Figure 2 shows an example of a PSD of an EEG signals and their bands.

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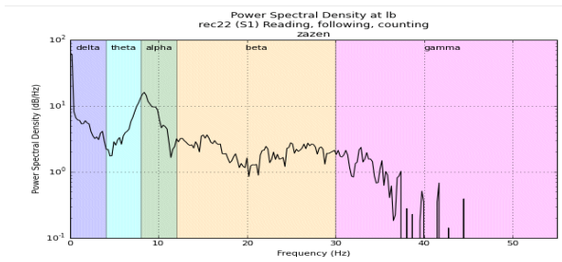

Figure 2 EEG Power Spectral Density
Source: (Trowbridge, 2015)

Coherence

Coherence is a frequency function given in normalized units between 0 and 1, that shows how much one signal *x(t)* corresponds in power to another signal *y(t)* for each frequency. In other words, coherence is a quadratic correlation coefficient that estimates the amplitude and phase consistence between two signals for every frequency. When the signal *x(t)* entirely corresponds to the signal *y(t)*, that means that are the same signal. Coherence is given by

$$\Gamma^2(\mathrm{f}) = \frac{\left|S_{xy}(\mathrm{f})\right|^2}{S_{xx}(\mathrm{f})S_{yy}(\mathrm{f})}; \quad 0 \le \Gamma(\mathrm{f}) \le 1. \tag{3}$$

Any pair of signals can be coherent in some frequency bands and have no coherence in another bands. Contrasting with amplitude measurements, coherence is the measurement of the synchronization between two signals principally based in the phase consistence. This represents that if two signals have different phase, like in simple linear circuits, high coherence is presented when the phase difference tends to remain constant.

For each frequency, the coherence measures when the signals are related by a linear time invariant transformation, which means that they have a constant amplitude and phase displacement rate (David et al., 2016; Schwartz, Kessler, Gaughan, & Buckley, 2017) (David et al., 2016) (Esqueda Elizondo José Jaime, Bermúdez Encarnación Enrique Guadalupe, Jiménez Beristáin Laura, Rojo Ramírez Yesenia, Ruiz Morales Angélica, Munguía Carrillo Paul Eriel, 2015).

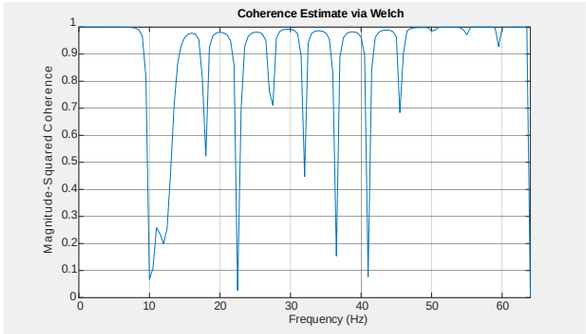

Figure 3 Coherence of two one seconds segment of an EEG signal
Source: Self-Made

Discrete Cosine Transform

The Discrete Cosine Transform (DCT) is a variant of the Discrete Fourier Transform (DFT), in which the signal is decomposed in a sum of cosine functions (not in sine and cosine functions as in the FDT) and these cosine coefficients are the interested features that describe the signals. The Discrete Cosine Transform is defined as:

$$C(u) = \alpha(u) \sum_{x=0}^{N-1} f(x) \cos\left(\frac{(2x+1)u\pi}{2N}\right) \tag{4}$$

where

$$\alpha(u) = \begin{cases} \sqrt{\frac{1}{N}} & para\ u = 0 \\ \sqrt{\frac{2}{N}} & para\ u = 1, 2, \ldots N-1 \end{cases} \tag{5}$$

The DCT is usually used to compress information because of its energy compaction property (Krishnan & Athavale, 2018) (Golmohammadi, Torbati, de Diego, Obeid, & Picone, 2017). Figure 4 shows an example of a DCT Power Spectrum of 3 128 samples sinusoidal signals with an offset.

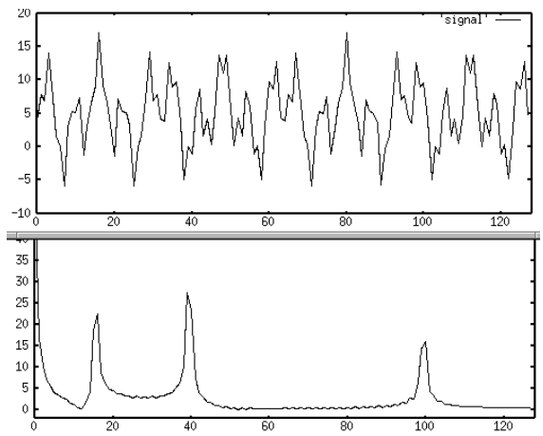

Figure 4 DCT Power Spectrum of three 128 samples sinusoidal signals with offset
Source: (Ruye Wang, 2007)

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Wavelet Transform

The Wavelet Transform is a mathematical tool that can carry out a Time-Frequency analysis of a signal. This is an advantage compared with Discrete Fourier Transform based techniques because with Fourier we can know which frequential components are present in the signal, but we do not know in which moment they appeared. With Wavelet Transform provides that information.

The Wavelet Transform is defined by:

$$W_f(s,\tau) = \int f(t)\psi^*_{s,\tau}(t)dt \quad (6)$$

Where $\psi^*_{s,\tau}(t) = \frac{1}{\sqrt{s}}\psi\left(\frac{t-\tau}{s}\right)$ is the mother Wavelet.

The Continuous Wavelet Transform is used in EEG signals to reduce noise in order to obtain a clearer and more precise signal. It can also be used to observe the Time-Frequency relationship of a signal. This Transform shows the moments with higher power in function of both time and frequency, representing them graphically in scalograms. The scalograms are a representation of the Wavelet Transform that in the *x* axis shows time and in the *y* axis shows the frequency scale and is given by:

$$\iint |CWT(\tau,\alpha)|^2 \frac{d\tau,d\alpha}{\alpha^2} = E_x \quad (7)$$

Where $E_x = \int |x(t)|^2 dt$

For a signal x(t), if the FDT is given by the relation $W(t,\omega)$, so the total energy of the signal will be:

$$\iint W(t,\omega) = \int |x(t)|^2 dt = \int |X(\omega)|^2 d\omega \quad (8)$$

Figure 5 shows both the time domain EEG signal and its Continuous Wavelet Transform, with the colormap, where red color means high power and blue color means low power.

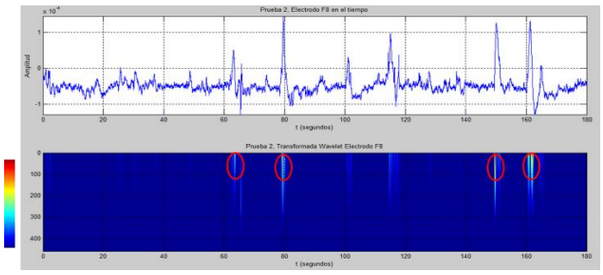

Figure 5 Time domain EEG signal and its Continuous Wavelet Transform
Source: Self-Made

Average Instant Frequency

The average instant frequency is given by the first invariant time movement of the FDT trough the frequency axes:

$$E_t\omega = \frac{1}{|x(t)|^2}\int \omega W(t,\omega)d\omega \quad (9)$$

And the group delay through the time axis is:

$$E_\omega t = \frac{1}{|X(\omega)|^2}\int tW(t,\omega)dt \quad (10)$$

In a similar way, the instant bandwidth is:

$$\sigma_\omega = \left(\int(\omega - \langle\omega\rangle)^2|X(\omega)|^2\right)^{1/2} \quad (11)$$

Where $\langle\omega\rangle = \int \omega|X(\omega)|^2 d\omega$.

Entropy

The entropy is a parameter that measures the randomness of a chaotic system or signal. Due to the complexity and non-linearity of the EEG signals, entropy can be used to analyze them. There are different types of entropy. For example, Shannon or log-energy entropies are used to measure the irregularities of EEG signals. Shannon entropy (H_{SE}) measures the average information contained in the probability distribution function (PDF). Log-energy entropy (H_{LE}) describe the electro-physiological behavior of different kinds of EEG signals. The Renyi entropy (H_{RE}) has been used to derive the continuous family of information measures. The Tsallis Wavelet entropy (H_{TE}) can extract some improved features by reducing the negative effects of the wavelet aliasing.

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For an N samples dataset, these entropies (Das & Bhuiyan, 2017) are given by:

$$H_{SE} = -\sum_{k=1}^{N} p_i^2 \times log_2(p_k^2) \quad (12)$$

$$H_{LE} = \sum_{k=1}^{N} log_2(p_k^2) \quad (13)$$

$$H_{RE} = \frac{1}{1-\propto} \, log \sum_{k=1}^{N} p_k^{\propto} \quad (14)$$

$$H_{TE} = \frac{1}{\propto -1}(1 - \sum_{k=1}^{N} p_k^{\propto}) \quad (15)$$

The Renyi entropy and the Tsallis entropy are of order α, where $\alpha > 0$ and $\alpha \neq 1$. If $\alpha = 2$, the estimation equally emphasizes the sub-Gaussian and the super-Gaussian components and is known as quadratic Renyi entropy and quadratic Tsallis entropy and they are given by (Das & Bhuiyan, 2017):

$$H_{RE} = -log \sum_{k=1}^{N} (p_k)^2 \quad (16)$$

$$H_{TE} = 1 - \sum_{k=1}^{N} p_k^2 \quad (17)$$

Machine Learning

Machine learning, also known as automatic learning is defined as the use of algorithms for data organization, pattern recognition in order for the computer to learn with these models and solve tasks without pre-programming. Machine learning is the computer's science area that defines Artificial Intelligence. The machine learning algorithms learn from the input data, and with these data, the machines are trained in order to execute different tasks autonomously.

Deep learning is the part of the automatic learning that using high level algorithms, imitates the neural network of the brain. These are complex algorithms made from several layers of neurons fed by a huge volume of data and are capable of image and speech recognition and to execute advanced tasks without human intervention (Krishnan & Athavale, 2018; Teo & Chia, 2018).

Experiences using Epoc BCI

During this research we worked with the Epoc+ made by the Emotiv Co. One of the lessons we learned with this project is that when we work with kids with Autistic Spectrum Disorder (ASD), first we have to spend some time trying to make the ASD user accepts and tolerates the headset.

We recommend that a visual inspection always be done first in order to eliminate and always record a video of the EEG recording process to have a timeline that can be used to locate movements that can generate disturbances. The problem with the new version of the Epoc+ is that a purchased software license is required in order to have access to the EEG data records.

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Conclusions

There are several techniques used to extract features from the EEG signals, depending on the intended use of the EEG signals. The literature reviewed recommends to combine feature extraction techniques and to extract features in different frequency bands, to have a more complete set of signal characteristics.

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Subprotestic stomatitis caused by incorrect choice of fixed protesis treatment

Estomatitis subprotestic causada por una elección incorrecta del tratamiento de protesta fijo

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Abatract

Fixed oral prosthesis as of late has been a very viable option for oral rehabilitation, since these differ from removable prostheses in aesthetic, functional and biological parameters. As a result, they are always preferred by patients. Oral prosthesis´ have certain parameters to be fulfilled for its prosthetic indications, such as the number of teeth present in the mouth, the type of occlusion, the level of bone insertion in dental organs that will be used as pillars or stumps, the proportion of root to crown , the absence of periradicular lesions that put us in reserved forecasts for the rehabilitation of dental organs. Under the ideal parameters we will base the final diagnosis for the placement of the correct restoration either in the same prosthetic design, manufactured with an ideal material. The preservation of the residual edge in the edentula area will be respected so that the prosthesis does not play an irritating role within the oral cavity, a relevant factor when placing this type of restorations. A bad adjustment either in individual crowns, fixed bridges can chronically irritate the residual flange resulting in ulcerative, fungal or bacterial lesions. Biological thicknesses, residual flanges must always be respected when designing this type of definitive restorations.

Prosthesis, Edentules, Dental Organs

Resumen

La prótesis bucal fija en la actualidad ha sido una opción muy viable para la rehabilitación bucal, ya que estas difieren de las prótesis removibles en parámetros estéticos, funcionales y biológicos. Dando como resultado que siempre son las preferidas de los pacientes. En la actualidad la prótesis bucal tiene ciertos parámetros a cumplir para su indicación protésicas, tanto como numero de dientes presentes en boca, el tipo de oclusión, Niveles de inserción ósea en órganos dentarios que se usaran como pilares o muñones, la proporción de corona raíz, la ausencia de lesiones periradiculares que nos pongan en pronósticos reservados la rehabilitación de órganos dentarios, bajo los parámetros ideales basaremos el diagnostico final para la colocación de la restauración correcta ya sea en el mismo diseño protésico, fabricado con un material ideal . La preservación del borde residual en la zona edentula se respetara para que la prótesis no juegue un papel irritativo dentro de la cavidad oral factor relevante a la hora de la colocación de este tipo de restauraciones.Un mal ajuste ya sea en coronas individuales, puentes fijos pueden irritar de manera crónica el reborde residual dando como consecuencias lesiones ulcerativas, micóticas o bacterianas. Siempre se deben respetar espesores biológicos, rebordes residuales cuando se diseñan este tipo de restauraciones definitivas.

Protesis fija,Edentulos, Órganos Dentarios

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† Researcher contributing as first author.

Introduction

The absence of one or more teeth involves a deficit in chewing efficiency with both functional and organic consequences, so it is important to avoid their loss and in the worst case try to replace them. The loss of teeth is due to various causes. Periodontal disease and caries are the most frequent[1], but also the agenesis absence of tooth formation, the lack of eruption, dental inclusion, trauma and tumors may make it necessary to replace them.

By replacing the teeth we also contribute to improving the aesthetics of the patient, since the absence of these, especially the former, produces a sinking of soft tissues and in many people produces an important unsightly effect. Subprothetic stomatitis is considered one of the most frequent pathologies (56%) that affect the oral tissues of subjects with total or partial removable dentures, 2-3 being more frequently found in the upper jaw2. The high frequency of patients with this pathology could be related to the presence of factors such as the poor hygiene of the prosthesis, the longer use of it, internal and contour irregularities, stability, retention, adaptation and occlusion of the prosthesis.

Causes of Dental Absences

In addition to trauma (blows), which can cause the loss of one or more teeth, the loss of teeth usually occurs due to two main causes:

A periodontal disease in its most advanced stage destroys the tissue that supports the tooth, so the teeth will end up moving and falling.

It can also be caused by caries so severe that it is not possible to save the tooth with a root canal. In that case, it is necessary to remove the dental piece to relieve the patient's pain and prevent the infection from spreading. Tooth loss, lose teeth.

Consequences of Dental Absences

A person with dental absences cannot chew food well, so it should be limited to soft foods, and the discomfort that this generates. In addition, with the deterioration of its masticatory function, a whole range of digestive and nutritional problems is exposed.

Changes in chewing mechanics can also cause ear, headache or cervical problems, but today the consequences are mostly psychological. Losing a tooth, especially on the aesthetic front, the visible part of the smile can cause real problems of self-esteem, in a society like today, which values youthful and healthy aspects so much. In fact, several studies of American, Japanese universities and Australians have concluded that the patients who solved their dental absences with osseointegrated implants felt much more confident and were happier in their daily lives than before solving their dental absence.

Treatment of Dental Absences Prosthetically

Dental absences used to be treated with removable dentures and dental bridges (dentures of a lifetime), but these solutions have clear limitations. These resin prostheses (or metal and resin) adhere poorly to the gum, which causes them to become unfit when the patient eats, talks or laughs.The best treatment for absences are dental implants. They consist of a titanium or zirconium screw, which integrates perfectly into the jaw bone and jaw, on which a crown is placed. With a dental implant, the patient will never notice a difference with a natural tooth. There is nothing that completely replaces a natural tooth, but an osteointegrated implant is as close as possible.

Objective

Know the different types of pontic used in PBF, the aesthetic, biological and functional advantages of the different types of pontic, as well as the most appropriate indication for your choice.

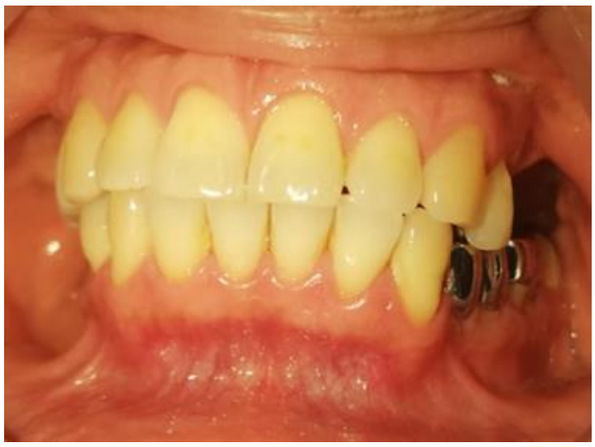

Figure 1

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Case Presentation

This is a 52-year-old male patient, attending clinic 2 of the faculty of dentistry Uac 2019. During the extraoral examination there were no facial alterations. or ganglionic, intraoral examination found various types of restorations including a metal bridge of OD 45-46-47, the patient referred to chewing pain in said restoration, for this reason the prosthesis was removed, when removed found in the residual flange zone a chronic ulcerative lesion approximately 2.5 cm in diameter below the pontic which was designed in saddle.

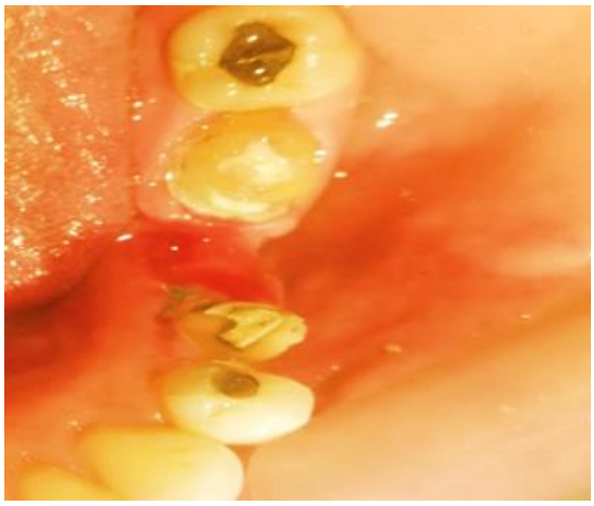

Figure 2

Diagnostic Methods Used
Differential Diagnoses

1. Traumatic Ulcer
2. Subprotic Stomatitis
3. Subprotic Mycosis

Clinical diagnosis

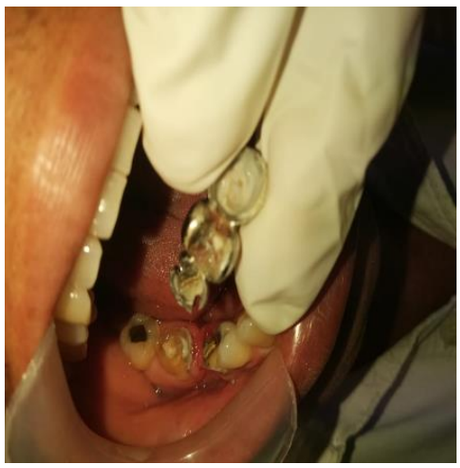

Figure 3

Radiographic Diagnosis

Facial alterations. or ganglionic, intraoral examination found various types of restorations including a metal bridge of OD 45-46-47, the patient referred to chewing pain in said restoration, for this reason the prosthesis was removed, when removed found in the residual flange zone a chronic ulcerative lesion approximately 2.5 cm in diameter below the pontic which was designed in saddle.

Initial Patient Status

Extraoral Analysis

Figure 4

It was observed that the ulcerative lesion was presented by the irritating factor that was the saddle pontics that had been placed since it had direct contact with the antagonist occlusion of the first upper molar causing this dental organ to generate continuous pressure towards the pontic during the masticatory cycles.

Dx: Sub prosthetic stomatitis

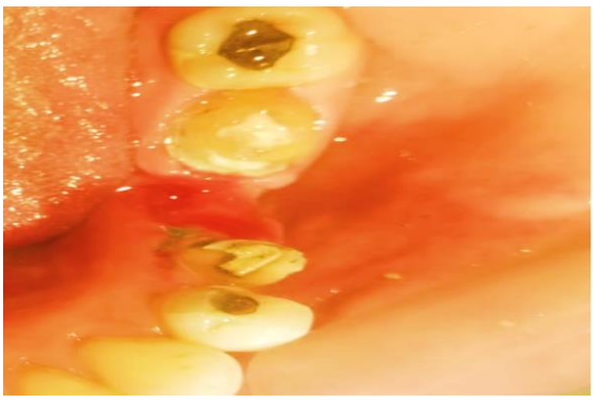

Figure 5

Periapical radiography Pos removal of the fixed bridge from 0D 45 - 47. The presence of injury is noted radiolucidal to OD 45 Pulp diagnosis: Coagulation necrosis and in periapical diagnosis: Asymptomatic chronic apical periodontitis.

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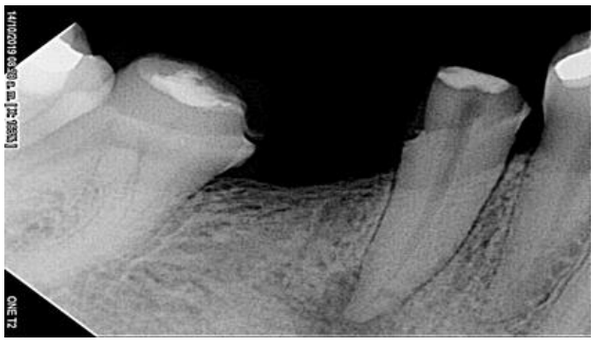

Figure 6

Treatment

Eliminate the irritating factor permanently and make a fixed oral prosthesis that suits the case. It was decided to make a bridge of 3 units with a hygienic pontic which in this case as a separation greater than 11 mm from the gingival edge to the antagonist tooth, it was the ideal choice for rehabilitation. It began with the impression of study models and assembly in semi-adjustable articulator for the preparation of a functional biological prototype that best suits the residual edge so as not to irritate the latter. Diagnostic waxing for the functional biological prototype

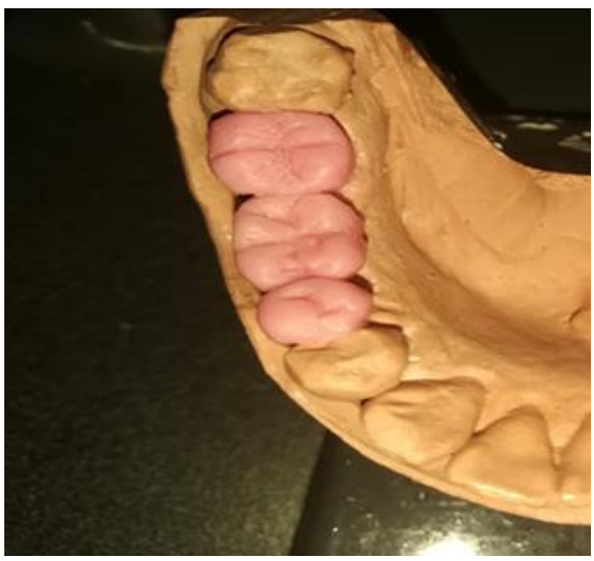

Figure 7

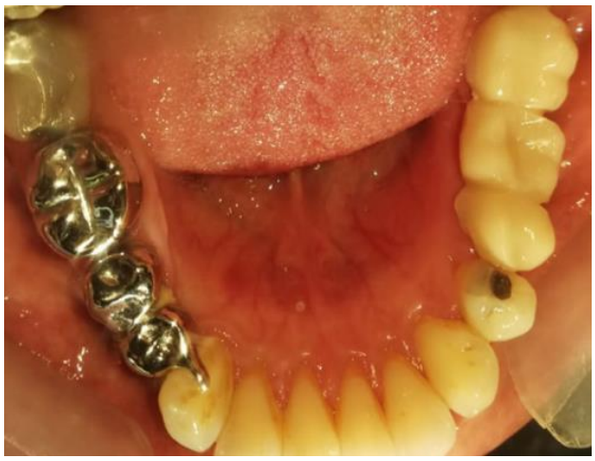

Figure 8

Occlusal adjustment of the PBF with semi-adjustable articulator. Manufacturing of the PBF in acrylic and adjustment in the mouth - 5 days after the removal of the fixed bridge

Figure 9

Monitoring

The patient was inspected 14 days after the installation of his provisional which was fixed to the mouth with a provisional cement that did not irritate the gum adjacent to the pillars and the edentulous space opted for the use of TEMB BOND (VOICO®) When removing the PBF An improvement in the healing of the ulcerative lesion was observed so that it recovered the continuity of the epithelium by 90% of its original surface.

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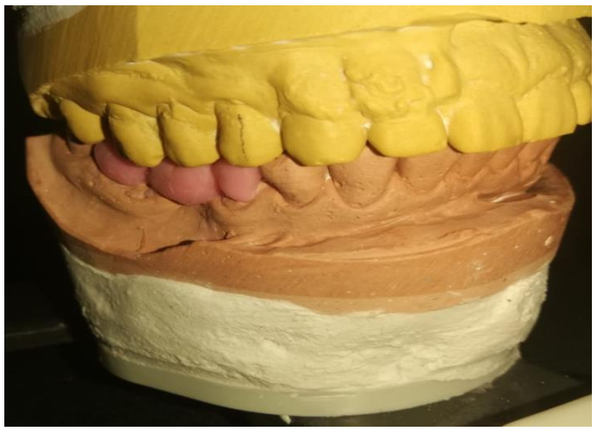

Figure 10

Pbf Manufacturing Protocol.

1.- A petroleum jelly surface is placed on the bridge pillars 2.- The Speedex® condensation silicone matrix was placed with enough Nic-Tone® color A2 self-healing acrylic in its filamentous phase.3.- The matrix is placed in the bridge area for this to end polymerize. 4.- Once polymerized, the acrylic surpluses are trimmed. Carbide stones and diamond discs for polishing with coarse-grained Green stones at 10,000 rpm. 5.- The polishing was done with Blankets, Pulecryl and white Spain at 10,000 rpm. PBF installation in mouth

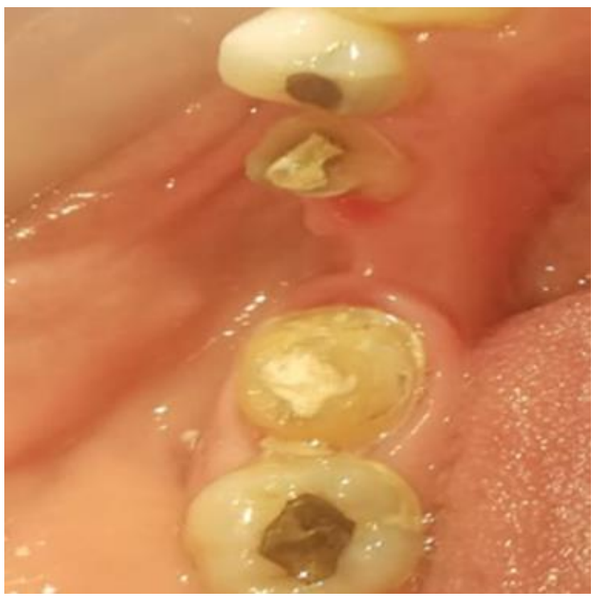

Figure 11

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Article **ECORFAN Journal-Ecuador**
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Synchronization of jealouses in simowal cows using Gnrh and PGF2α in the Conception, Michoacán

Sincronización de celos en vacas simmental utilizando GNRH y PGF2α en la Concepcion, Michoacán

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Abstract

The objective of this work is to reduce the time of presentation of jealousy and improve the indices of conception. The synchronization of estrus or estrus is a technique that is used to achieve the greatest number of pregnant females. GnRH stimulates the release of FSH and LH. FSH promotes follicular growth which increases the concentration of estrogens by the growth of the follicles. In this experiment, 6 cows of the Simmental breed were synchronized with a range of 0 to 7 births, natural riding was provided with a stallion of the same breed. The adoption of synchronization protocols has been increasing today due to the high market demand. A 66.6% favorable response to treatment was obtained. 33.3% presented estrus at 26 hours and the other 33.3% presented estrus at 30 hours after day 10. Understanding the reproductive physiology of the female and the male allows us to develop synchronization protocols, GnRH and PGF2α, are the methods what better results are producing in our races,

Protocol, Synchronization, Zeal

Resumen

El objetivo de este trabajo es reducir el tiempo de presentación de celos y mejorar los índices de concepción. *La sincronización de celos o estros es una técnica que se emplea para lograr el mayor número de hembras gestantes.* La GnRH, estimula la liberación de FSH y LH. La FSH promueve el crecimiento folicular lo que aumenta la concentración de estrógenos por el crecimiento de los folículos. En este experimento, se sincronizaron 6 vacas de la raza Simmental con un intervalo de 0 a 7 partos, se proporcionó monta natural con un semental de la misma raza. La adopción de protocolos de sincronización ha ido aumentando hoy en día debido a la gran demanda del mercado. Se obtuvo un 66.6% de repuesta favorable al tratamiento. El 33.3% presentó estro a las 26 horas y el otro 33.3% presento celo a las 30 horas después del día 10. Comprender la fisiología reproductiva de la hembra y el macho nos permite desarrollar protocolos de sincronización, la GnRH y PGF2α, son los métodos que mejores resultados están produciendo en nuestras razas,

Protocolo, Sincronización, Celo

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Introduction

The synchronization of estrus involves the control or manipulation of the estrous cycle so that the females chosen in a herd express estrus (heat) at approximately the same time. It is a technique widely used in artificial insemination programs, embryo transplantation, birth concentrations and natural mounts. The determining factor in the success of the synchronization is the choice of the appropriate method, which adjusts to the conditions of each animal (Córdoba, 2011).

Synchronization consists in the application of a hormonal product. GnRH or also called gonadotropin-releasing hormone, stimulates the release of FSH and LH. FSH promotes follicular growth which increases estrogen concentration by follicle growth. Subsequently, increasing the level of estrogen stimulates the release of LH which occurs in the form of a peak. LH influences ovulation, when ovulation begins, also does the follicle leutinization process. (Becaluba, 2016).

PGF2α is a hormone that is naturally produced by the endometrium and acts in the last period of the cycle causing the corpus luteum to regress and resume the next cycle (Colazo, 2007).

The advantages of estrous synchronization (ES) are multiple: it facilitates the implementation of artificial insemination (AI), increases the rate of birth and weaning, reduces the interval between births, programming of females so that they give birth at certain times, geneticall improvement of the herd (Vallejo, 2015).

The signs of heat and their detection play an important role since the percentage of cows in estrus is determined from this. The behavior of the cow in heat generally lasts for 25 hours; This average varies between cows. Physical and behavioral changes are observed; the vulva becomes edematized by the increase of blood to the reproductive organs, discharge of mucus from the vulva, nervousness and excitement, muted, the cows will ride to other cows although a cow in real heat is allowed to ride by another, fold the chin and they lick the flanks of another cow, some walk two or three times more than normal, are grouped into sexually active animals.

The ovule is released approximately 6 to 12 hours after heat. The ovule is detached from the follicle and can be penetrated by the sperm; by this time, it must be ensured that the semen is already inside the cow (Guaqueta, 2009)
There are two basic observation methods for detecting heat; One is the visual observation where two or more periods of the day are selected to observe and detect the cows in heat, the other method consists of constant observation using video cameras that show the day and night activity. Observation is very important, and it needs enough and exclusively time to observe all animals, 70% of heat will take place between 7:00 pm at 7:00 am. (Marcantonio, 1998).

Feeding also plays an important role in the reproductive cycle, for example any imbalance in the Na: K ratio will affect the estrous cycle, directly and indirectly, through the alteration in protein synthesis and the loss of water regulation, an important factor for the secretions that must accompany the manifestation of heat. On the other hand, the consumption of crude protein or energy deficiency increases urea in the blood and produces tissue toxicity, affecting the endometrium and decreasing the production of prostaglandins, responsible for lysing the corpus luteum, which, if not lysed, causes a long estrous cycle , it can be explained at the hypothalamus-pituitary axis level, in which, low glucose levels inhibit the secretion of GnRH and cause a decrease in LH pulsatility (Campos, 2009).

Finally, an additional factor that induces alteration of estrous cyclicity is phytoestrogens, plants with high lsoflavone contents that have an estrogenic action in the animal organism.

Phytoestrogens are widespread, in clover, alfalfa, corn and intensively grown grasses, they are different from estrogens produced by the animal, but their action is identical on the receptors of the reproductive tract; its metabolization and excretion is different (for example demetilization) and its renal excretion through glucoronosides, likewise the formation of sulfate esters in liver tissue is lower, accumulating in circulation and altering the estrogen of the cycle, this leads to nymphomania of the cows and with it the loss of reproductive function.

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Materials and methods

The work was carried out, in the town of La Concepción located in the municipality of Morelia in the state of Michoacán de Ocampo and is located at the coordinates: Longitude (dec): - 101.309722, Latitude (dec): 19.7033611. It is located at a height of 2140 meters above sea level. It was held in June of this year.

We worked with a total of 6 cows of the Simmental breed with a range of 0 to 7 deliveries in order to present estrous cycle on day 10. On the other hand, the natural riding was carried out with a bull of the same breed.
The protocol (Ovsynch) with which this experiment was carried out was based on the luteolytic effect of prostaglandins (PGF2α), on the effect of progestogens to inhibit estrus behavior, as well as follicular control and luteum with releasing hormone of gonadotropins (GnRH) in combination with (PGF2α).

The first application of GnRH was given to induce ovulation and promote the formation of a new corpus luteum (CL) and a new follicular wave; that is, to return the cow "at the beginning of the estrous cycle". The prostaglandin used on day 7 was to return the new CL and the last GnRH was administered on day 9 in order to induce ovulation of the new follicle. The reasoning of this protocol is to give one more day for follicular growth that may allow further maturation of the oocyte and ovulation of a larger follicle (López, 2007).

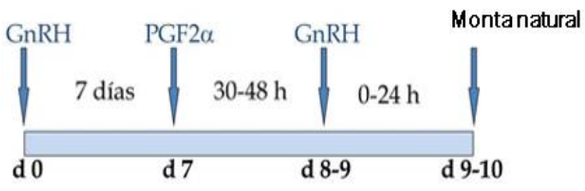

Figure 1

The applications of the injections, in the case of GnRH, were administered (2ml per day 0 and 5ml per day 9) subcutaneously in the neck table with con inch needle; which consists of the introduction of medications into the subcutaneous tissue; it is applied when it is desired that the medicine be absorbed slowly which allows to ensure a sustained effect.

PGF2α was applied (5ml per day 7) intramuscularly in the leg with a 1½ inch needle; which consists of the administration of medicines in the muscular tissue, used mainly in those cases in which a greater rapidity is required; The rate of absorption depends on factors such as muscle mass at the injection site and blood supply. As mentioned, the detection of jealousy in a herd plays an important role since it allows indirectly to increase monetary gains due to a good observation of estrus and therefore pregnant cows which in the future the calf will represent monetary income to a production unit Therefore, for the detection we use the method of visual observation which consisted of 3 times for 12 hours a day 10.

Results and discussion

The synchronization was carried out in 6 cows of which 66.6% of the favorable response to the treatment was obtained. 33.3% had estrus at 26 hours and the other 33.3% had heat at 30 hours after day 10. The results obtained are similar to those found by (López, 2007) who used the Ovsynch protocol, obtaining a 68.75% heat manifestation at the time of the second application of GnRH.

Among the alternatives to contribute to an improvement in the reproductive efficiency of cattle, the use of jealousy synchronization protocols is considered since the investigations carried out show that the hormonal manipulation of the ovarian cycle of cattle with good results is feasible, in addition, it allows to have better control in the herd for an artificial insemination program.

Conclusions

The process of synchronization of the females so that they enter in heat at a certain moment allows the Artificial Insemination in Fixed Time (IATF) favoring the use of the Artificial Insemination (AI) on a large scale or the natural mount, and diminishes the labor force necessary to monitor females in heat. The adoption of synchronization protocols has been increasing today due to high market demand.

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The greater understanding of the function of the reproductive system of the female and the male has allowed us to understand how the synchronization protocol already performed develops and how it acts in the herd, as well as the understanding of bovine reproductive physiology allows us to choose the protocol of most appropriate synchronization for each herd. It is worth mentioning that good management, nutrition and attention to detail are extremely important for success..

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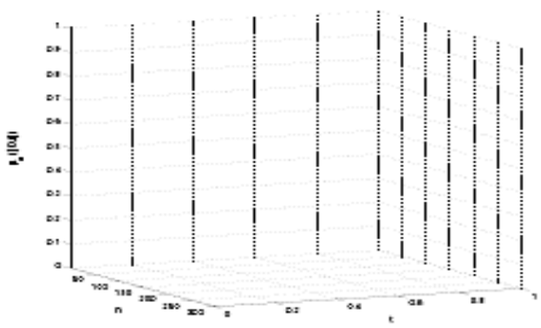

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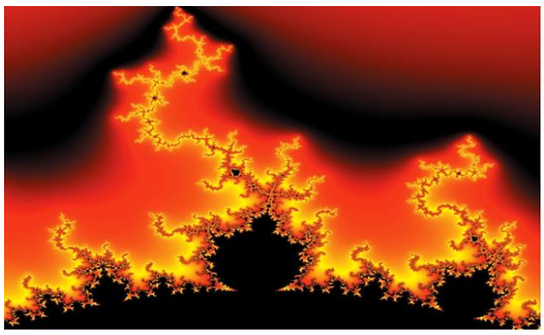

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